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Induced Cytochrome P450 in Winter Flounder (*Pseudopleuronectes americanus*) from Coastal Massachusetts Evaluated by Catalytic Assay and Monoclonal Antibody Probes[1]

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Stegeman, J. J., F. Y. Teng, and E. A. Snowberger. 1987. Induced cytochrome P450 in winter flounder (*Pseudopleuronectes americanus*) from coastal Massachusetts evaluated by catalytic assay and monoclonal antibody probes. Can. J. Fish. Aquat. Sci. 44: 1270–1277.

Levels of hepatic microsomal ethoxyresorufin *O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities and sensitivity to inhibition by α-naphthoflavone in winter flounder (*Pseudopleuronectes americanus*) from Deer Island flats in Boston Harbor, off Plymouth Beach, off Nantucket, and at the outer New Bedford Harbor in Buzzards Bay, Massachusetts, suggested induction of cytochrome P450 by environmental chemicals. Levels of activity were higher in fish from Boston Harbor and Plymouth Bay than from Nantucket and Buzzards Bay. In fish from Buzzards Bay the levels of EROD and AHH activities were closely correlated, with some fish there apparently uninduced. Immunoblot analysis of flounder liver microsomes with a monoclonal antibody (1-12-3) against β-naphthoflavone-inducible scup cytochrome P450E revealed a single cross-reacting protein in untreated fish from all four field sites. A similar protein was induced by β-naphthoflavone treatment. Levels of this flounder protein correlated positively with levels of AHH and EROD activity in Buzzards Bay fish, consistent with a conclusion that some fish there were uninduced. The results demonstrate the induction of a P450E counterpart in flounder in Massachusetts waters and indicate its identity as the AHH and EROD catalyst. The results also establish the use of monoclonal anti-P450E in analysis of environmental induction.

Les niveaux d'activité de l'éthoxyrésorufin *O*-dééthylase (EROD) et de l'hydrolase d'hydrocarbure arylé (AHH) ainsi que la sensibilité à l'inhibition par la α-naphthoflavone dans les microsomes hépathiques de la plie rouge (*Pseudopleuronectes americanus*) échantillonnée sur les sèches de l'île Deer dans le port de Boston, au large de Plymouth Beach, au large de Nantucket et dans la partie extérieure du port de New Bedford dans la baie Buzzards (Massachusetts) portent à croire à l'induction du cytochrome P450 par des produits chimiques environnementaux. Les niveaux d'activité étaient plus élevés chez les plies du port de Boston et de la baie Plymouth que chez celles de Nantucket et de la baie Buzzards. Chez ces dernières, les niveaux d'activité de l'EROD et de l'AHH étaient en corrélation étroite quoique apparemment certains poissons n'aient pas été induits. L'analyse immunologique des microsomes hépathiques de la plie à l'aide d'un anticorps monoclonal (1-12-3) en réaction avec le cytochrome P450E inductible par la β-naphthoflavone extraite provenant du spare doré a révélé une seule protéine à réaction croisée chez les poissons non traités provenant des quatre sites expérimentaux. On a induit une protéine semblable par traitement au β-naphthoflavone. Les teneurs en protéines tirées de cette plie étaient en corrélation positive avec les niveaux d'activité de l'AHH et de l'EROD chez les poissons de la baie Buzzards; ceci est en accord avec la conclusion que certains poissons échantillonnés dans cette baie n'étaient pas induits. Les résultats révèlent l'induction d'un analogue du P450E chez la plie des eaux du Massachusetts et l'identifient comme le catalyseur de l'AHH et de l'EROD. Les résultats démontrent aussi l'utilisation de l'anti-P450E monoclonal pour l'analyse de l'induction environnementale.

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Populations of fish in some coastal waters and rivers of North America have high incidences of hepatic and other neoplasms (Malins et al. 1985; Couch and Harshbarger 1985). Most recently, an epizootic of hepatic neoplastic and preneoplastic lesions was reported in winter flounder (*Pseudopleuronectes americanus*) near Deer Island in Boston Harbor, Massachusetts (Murchelano and Wolke 1985). The origin of these latter tumors is not known, but there are high levels of polycyclic aromatic hydrocarbons (PAH) in sediments near Deer Island (Boehm et al. 1984), and association between PAH and flatfish tumors has been described in other regions (Malins et al. 1985). Sediments in other coastal sites in Massachusetts have very high levels of other pollutants, particularly polychlorinated biphenyls (PCBs) (Farrington et al. 1983). Both types of compounds could affect winter flounder in these regions.

Oxidative metabolism by cytochrome P450 monooxygenases is a critical process determining the biological activity of many xenobiotics through compound activation or inactivation. The major form of P450 induced by PAH and PCBs has been identified in some teleost species, i.e. cytochrome P450E from scup (*Stenotomus chrysops*) (Klotz et al. 1983) and

[1]Contribution No. 6274 of the Woods Hole Oceanographic Institution. This article concerns the apparent fish counterpart of P450IA1, equal to rat P450c, mouse $P_1$450, or rabbit P450 6 (see Nebert et al. 1987; Stegeman and Kloepper-Sams 1987).

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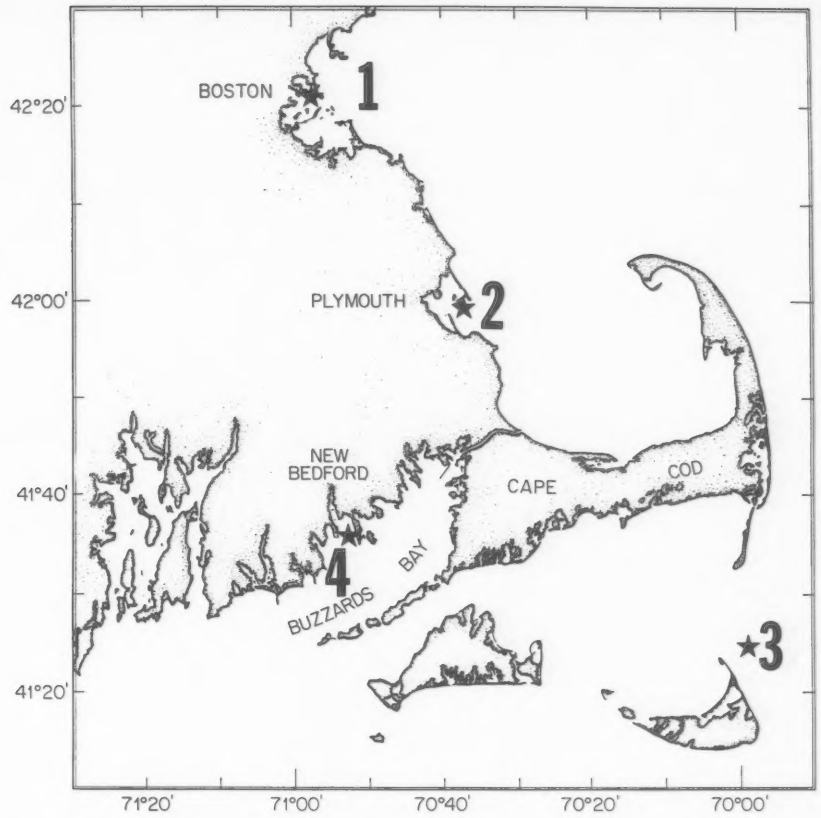

FIG. 1. Winter flounder sampling locations. Site 1, near Deer Island sewage outfall in Boston Harbor, June 25, 1982. This is the same site as that sampled by Boehm et al. (1984). Site 2, off Plymouth Beach, June 25, 1982. Site 3, Nantucket Sound, October 5, 1982. Site 4, Buzzards Bay, near Butler Flats, July 24, 1982.

cytochrome P450 LM_{4b} from rainbow trout (*Salmo gairdneri*) (Williams and Buhler 1984). These fish proteins are functional analogues of rat P450c (recently designated P450IA1; Nebert et al. 1987), catalyzing both aryl hydrocarbon hyroxylase (AHH) and ethoxyresorufin *O*-deethylase (EROD) activities, and the metabolism of benzo[a]pyrene (B[a]P) leading toward diolepoxide formation (Stegeman and Kloepper-Sams 1987). Cytochrome P450c in rats (Ryan et al. 1979) is the form primarily responsible for the formation of carcinogenic diolepoxides of some PAH (e.g. Jerina et al. 1984). The function of such proteins could be related to pathologies found in some fish from polluted waters. Induction of specific P450 forms could thus indicate exposure of populations to biochemically significant levels of xenobiotic inducers, and indicate elevated capacity to activate procarcinogens.

Here we describe the occurrence of induced P450 in winter flounder from sites off coastal Massachusetts, detected both by catalytic assays and immunochemical probes. Catalytic assays have previously been used to describe monooxygenase induction in winter flounder from coastal Maine (Foureman et al. 1983). Our purposes here were to determine whether a counterpart to P450E is the catalyst-induced in winter flounder from Massachusetts waters, including sites where tumor-bearing fish were taken and where there is contamination by PAH or PCBs. Further objectives were to evaluate the use of heterologous monoclonal antibodies in analysis of such induction.

Materials and Methods

Chemicals

[^{3}H]-Benzo(a)pyrene was obtained from the Amersham Corporation and 7-ethoxyresorufin was synthesized as before (Klotz et al. 1984). NADPH and NADH were from Sigma Chemical Co. (St. Louis, MO), and α-naphthoflavone (ANF) and β-naphthoflavone (BNF) were from Aldrich Chem. Co., Milwaukee, WI. Monoclonal antibody to scup P450E was obtained in ascites fluids as previously described (Park et al. 1986).

Animals

Winter flounder were collected by otter trawl between June and early October 1982 from locations indicated in Fig. 1. Average weight of fish from all sites was 321 ± 101 g. Fish from Boston Harbor and Plymouth Bay were the same animals employed in histological and biochemical studies by Carr and Hillman (Battelle Labs, Duxbury, MA, unpubl.). All fish used were outside of the normal gonadal maturation season, and

TABLE 1. Cytochrome P450 content and monooxygenase activity (mean ± SD) in treated winter flounder.

Component/activity	Control (N = 5)	BNF-treated (N = 6)
Cytochrome P450 (nmol·mg^{-1})	0.59 ± 0.28	0.76 ± 0.15
Fe^{2+}-CO λ_{max}	448.9 ± 0.4	448.4 ± 0.2
Cytochrome b_5 (nmol·mg^{-1})	0.05 ± 0.01	0.05 ± 0.02
EROD (units·min^{-1}·nmol P450^{-1})[a]	1.71 ± 0.37	2.78 ± 0.44[b]
AHH (units·min^{-1}·nmol P450^{-1})[a]	1.01 ± 0.26	1.37 ± 0.19[c]

[a]Units are nanomoles resorufin produced or nanomoles B[a]P metabolites produced.
[b]Different from control at $P \leq 0.02$.
[c]Different from control at $P \leq 0.05$.

none had developed gonads. Water temperatures at the time of collection were between 14 and 18°C. Animals were killed immediately after capture, and portions of liver were removed and held in liquid nitrogen for periods of 3–25 d before preparation of microsomal fractions.

Winter flounder weighing between 200 and 300 g were also collected from Menemsha Pond, Vineyard Sound, in May 1985 and used to verify the induction of a cytochrome P450E counterpart in this species. Prior to treatment, these fish were maintained for 1 mo on clean sediment in flow-through water of ambient temperature at the shore laboratory, Woods Hole Oceanographic Institution. Temperature at the time of treatment was 18°C, similar to that at sites of field collection. Fish were maintained on a diet of chopped squid fed ad libitum. Fish were injected intraperitoneally with 20 mg BNF·kg^{-1} in corn oil or corn oil alone on days 1 and 4 and were killed on the seventh day after initial injection; livers were removed and microsomal preparations were obtained as below.

Microsomal Preparations

Tissues were homogenized in 10 volumes of 0.25 M sucrose and 0.1 M Tris–HCl (pH 7.4) using a Potter–Elvehjem tissue grinder. Subcellular fractions were obtained by differential centrifugation as before (Stegeman et al. 1979). Microsomal pellets were resuspended in 3 volumes of 50 mM Tris–HCl (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol (pH 7.5) and stored in liquid nitrogen. In other studies, we have found microsomes stored under these conditions to retain full catalytic activity for a period of years (Stegeman 1987). Protein content was determined according to Lowry et al. (1951).

Enzyme Assays

AHH activity was assessed by measuring the production of water-soluble metabolites from [^{3}H]BP, based on the method of Van Cantfort et al. (1977). Reaction mixtures consisted of 50 mM Tris–HCl (pH 7.4), 0.88 mg bovine serum albumin·mL^{-1}, 0.40 mM NADPH, 60 μM [^{3}H]BP (approximately 590 × 10^4 Bq·μmol^{-1}), and about 3 pmol of microsomal cytochrome P450 in a volume of 50 μL. Some reaction mixtures were modified to include 0.1 mM ANF. Blank reactions consisted of reaction mixtures with boiled microsomes. Reactions were initiated by addition of NADPH and incubated at 30°C for 25 min. Assays were done in triplicate under red light, and metabolite formation was measured as previously described (Binder and Stegeman 1980).

EROD activity was measured by a method described previously (Stegeman et al. 1981; Klotz et al. 1984). The reaction mixture consisted of 0.1 M Tris–HCl (pH 8.0), 0.1 M KCl, 0.5 mM NADPH, 2 μM 7-ethoxyresorufin, and 5 μL of microsomes (5–16 mg protein·mL^{-1}) in a volume of 0.5 mL. Reactions at 30°C were initiated by adding 10 μL of NADPH and proceeded at 30°C for up to 5 min. Resorufin (ϵ_{572} = 73 mM^{-1} cm^{-1}) formation was monitored with a Cary 118C spectrophotometer.

Cytochrome P450 (ϵ = 91 mM^{-1} cm^{-1}) was measured by sodium dithionite difference spectra of CO-treated samples, and cytochrome b_5 content (ϵ = 185 mM^{-1} cm^{-1}) was determined from NADH difference spectra as previously described (Stegeman et al. 1979). Each cuvette contained approximately 1 mg microsomal protein·mL^{-1}.

Immunoblots

Immunoblot ("Western" blot) analysis was accomplished with monoclonal antibody MAb 1-12-3 against P450E, the major PAH-induced form isolated from the marine fish scup (Klotz et al. 1983). The characterization of this antibody and its specificity in immunoblotting has been described (Park et al. 1986; Kloepper-Sams et al. 1987). Microsomal samples were incubated in a steaming water bath for 10 min in 62.5 mM Tris–HCl (pH 6.8), 3% sodium dodecyl sulfate (SDS), 10% glycerol, and 5% β-mercaptoethanol. Proteins were electrophoretically resolved and were transferred onto 0.2-μm nitrocellulose paper essentially as described by Towbin et al. (1979). The blots were incubated in 5% dry milk in phosphate-buffered saline (PBS) for 1 h at 42°C and MAb 1-12-3 diluted in PBS for 2 h at room temperature. They were washed 10 min each in PBS, 0.5% Tween 20 plus PBS, and PBS again and then incubated for 1 h in PBS with 5 μL goat anti-mouse peroxidase-linked IgG·mL^{-1} and 5% dry milk. They were washed again as above and developed in HRP color developer (BioRad) containing 4-chloro-1-naphthol and 0.02% hydrogen peroxide in PBS for 30 min. Staining was evaluated by densitometric analysis with a soft laser densitometer (Helena Labs Inc.).

Results

Liver microsomes from BNF-treated winter flounder had levels of AHH and EROD activity that were significantly greater than those seen in the control animals (Table 1). This result and those of other studies (Foureman et al. 1983) provide a basis for interpreting data from field samples of this species. There were no apparent sex differences in levels of microsomal P450 or in monooxygenase activity in fish from the field

TABLE 2. Cytochrome P450 and monooxygenase characteristics (mean ± SD) in male and female flounder from Boston Harbor.

Component/activity	Males (N = 11)	Females (N = 9)
Microsomal yield ($mg \cdot g^{-1}$)	10.7 ± 2.0	12.8 ± 2.8
Cytochrome P450 ($nmol \cdot mg^{-1}$)	0.623 ± 0.24	0.627 ± 0.25
Cytochrome b_5 ($nmol \cdot mg^{-1}$)	0.056 ± 0.018	0.058 ± 0.021
EROD ($units \cdot min^{-1} \cdot mg^{-1}$)[a]	1.19 ± 0.48	1.20 ± 0.43
EROD ($units \cdot min^{-1} \cdot nmol\ P450^{-1}$)[a]	1.9 ± 0.8	1.7 ± 0.6

[a]Units are nanomoles of resorufin produced.

TABLE 3. Cytochrome P450 content and MFO activities (mean ± SD) in winter flounder.

Component/activity	Boston Harbor (N = 18)	Plymouth Bay (N = 18)	Nantucket Sound (N = 8)	Buzzards Bay (N = 8)
Microsomal yield ($mg \cdot g^{-1}$)	11.6 ± 0.6	12.4 ± 0.6	9.2 ± 1.1	16.1 ± 1.3
Cytochrome P450 ($nmol \cdot mg^{-1}$)[a]	0.62 ± 0.06	0.67 ± 0.07	1.04 ± 0.13	0.90 ± 0.13
Fe^{2+} CO λ_{max} (nm)	449.4 ± 0.1	449.0 ± 0.1	449.1 ± 0.1	449.5 ± 0.2
Cytochrome b_5 ($nmol \cdot mg^{-1}$)	0.06 ± 0.01	0.05 ± 0.01	0.11 ± 0.01	0.16 ± 0.02
EROD ($units \cdot min^{-1} \cdot mg^{-1}$)[b]	1.19 ± 0.11	1.27 ± 0.14	0.87 ± 0.12	0.78 ± 0.39
EROD ($units \cdot min^{-1} \cdot nmol\ P450^{-1}$)[b]	1.81 ± 0.16	1.95 ± 0.15	1.08 ± 0.21	0.64 ± 0.28
AHH ($units \cdot min^{-1} \cdot mg^{-1}$)[c]	0.59 ± 0.02[d]	0.62 ± 0.02[d]	0.77 ± 0.36	0.45 ± 0.63
AHH ($units \cdot min^{-1} \cdot nmol\ P450^{-1}$)[c]	0.78 ± 0.06[d]	0.80 ± 0.04[d]	0.74 ± 0.30	0.37 ± 0.39
% ANF inhibition[e]	87[d]	89[d]	93 ± 1	45 ± 16

[a]There was no quantifiable cytochrome P420 in any of these samples.

[b]Units are nanomoles resorufin produced. EROD activity per nanomole P450 was significantly lower in Buzzards Bay fish than that in Boston Harbor or Plymouth Bay fish, with $P = 0.005$, and lower in Nantucket Sound fish than in Boston or Plymouth, with $P = 0.02$. Data were analyzed by the Mann–Whitney U-test.

[c]Units are nanomoles B[a]P metabolites produced.

[d]Values determined from pooled samples.

[e]Indicates inhibition of AHH activity by 0.1 mM ANF.

(Table 2), relative to the profound differences occurring in gonadally (seasonally) mature winter flounder (Stegeman and Woodin 1984). Data for fish from Boston Harbor are shown (Table 2), but similar results were obtained for fish from the other sites. The absence of sexual differentiation is consistent with sampling during summer to early fall when gonadal function and hormonal distinctions between the sexes are minimal.

Catalytic Functions

The specific content of hepatic microsomal P450 was higher in fish from Nantucket Sound and Buzzards Bay than in animals from Plymouth Bay and Boston Harbor (Table 3); the relative levels of cytochrome b_5 paralleled those of P450. Levels of microsomal EROD activity were appreciable in fish from all four sites. Activity was highest in fish from Boston Harbor and Plymouth Bay, about 2 $nmol \cdot min^{-1} \cdot nmol\ P450^{-1}$. The average level of EROD activity in Nantucket Sound fish was about half that seen in Boston Harbor fish, and in fish from Buzzards Bay the mean activity was still lower, 0.64 $nmol \cdot min^{-1} \cdot nmol\ P450^{-1}$. The range in levels of EROD activity was greatest in Buzzards Bay fish, extending from nondetectable up to 2.0 $nmol \cdot min^{-1} \cdot nmol$ microsomal $P450^{-1}$.

Levels of AHH activity averaged 0.75 $nmol \cdot min^{-1} \cdot nmol\ P450^{-1}$ in fish from Nantucket Sound, Plymouth Bay, and Boston Harbor (Table 3). Activities in these fish were inhibited to a similar degree by 0.1 mM ANF, which has been demonstrated to inhibit the major PAH-induced cytochrome P450 isozyme in fish (Klotz et al. 1983). The mean AHH activity determined in fish from Buzzards Bay was about half that seen in fish from the other sites. The degree of inhibition by ANF was also less in fish from Buzzards Bay, and one individual in that group had activity that was stimulated by ANF.

There was a strong correlation ($r^2 = 0.98$) between the levels of EROD and AHH activities in the winter flounder collected from Buzzards Bay (Table 4). Animals having little or no detectable EROD activity also had low AHH activity that was only slightly inhibited or was stimulated by ANF. The Fe^{2+}-CO absorption maximum of microsomal P450 in those individuals was at 450 nm. The animal with the highest EROD activity also had the highest AHH activity, which was strongly inhibited by ANF, and a microsomal Fe^{2+}-CO absorption maximum at 448 nm, properties consistent with those typical of PAH-induced P450 forms. The transition from stimulation to inhibition of flounder microsomal AHH by ANF occurs at very low levels of activity, i.e. very low levels of induced protein, consistent with earlier results (Foureman et al. 1983). In contrast with the Buzzards Bay fish, animals from Nantucket Sound did not show any significant correlation between levels of EROP and AHH activities or between levels of either of these activities with any other characteristics.

Immunochemical Data

The above data suggest the presence of induced cytochrome P450 in fish from each of the four sites. The nature of this

TABLE 4. Microsomal properties of winter flounder from Buzzards Bay and Nantucket Sound.

Individual	EROD (nmol·min^{-1}·nmol P450^{-1})	AHH	% ANF inhibition[c]	Fe^{2+}-CO λ_{max}
		Buzzards Bay[a]		
1	<0.09[b]	0.04	−37	450.0
2	<0.06[b]	0.03	−15	450.0
3	<0.08[b]	0.08	15	450.0
4	0.25	0.22	48	450.0
5	0.40	0.19	89	449.7
6	1.09	0.68	89	449.5
7	1.23	0.65	90	449.5
8	1.98	1.08	93	448.0
		Nantucket Sound		
9	0.32	0.68	94	449.0
10	0.47	0.75	92	449.0
11	0.68	1.02	93	449.5
12	0.97	0.58	91	449.5
13	1.04	0.33	93	449.0
14	1.38	1.31	93	449.0
15	1.68	0.52	93	449.0
16	1.88	0.77	95	449.0

[a]Levels of AHH and EROD activities in fish from Buzzards Bay were correlated with $r^2 = 0.98$, significant at $P = 0.01$.
[b]Limits of detection.
[c]Negative value indicates stimulation of AHH activity by 0.1 m*M* ANF.

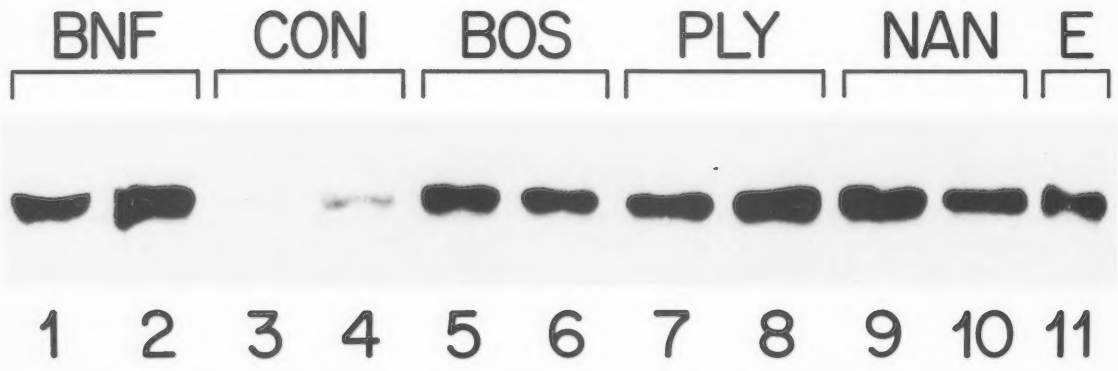

FIG. 2. Immunoblot of winter flounder hepatic microsomes. Lanes 1−10 contained 8 pmol of microsomal P450 from two samples each of BNF-treated, control, Boston Harbor, Plymouth Bay, and Nantucket Sound winter flounder, respectively. The same amount of microsomal cytochrome P450 was applied to each lane so that content of cross-reacting protein as a percentage of the total cytochrome P450 would be evident. Lane 11 contained 3.0 pmol of purified scup cytochrome P450E.

cytochrome P450 was evaluated by analysis with the monoclonal antibody MAb 1-12-3 against cytochrome P450E, the major AHH and EROD catalyst from scup. Immunoblotting of liver microsomes (Fig. 2) showed recognition of a single protein band, about 55−56 kD, which was present at high levels in BNF-treated fish but was barely detectable in control fish. A similar cross-reacting protein was abundant in liver microsomes of all fish analyzed from Boston Harbor, Plymouth Bay, and Nantucket Sound (Fig. 3).

In Buzzards Bay fish, the ranges seen in levels of microsomal P450, optical properties, and catalytic activities suggested a range in levels of an induced P450 isozyme. Analysis of these samples by immunoblot (Fig. 3) showed marked individual differences in amount of the protein recognized by MAb 1-12-3. There was a positive correlation between the relative amount of the cross-reacting protein and the levels of EROD and AHH activity, with $r^2 = 0.92$ or better (Fig. 4). The affinity of the MAb 1-12-3 for the flounder counterpart of scup P450E is not known. Assuming affinity similar to that for P450E, then the P450E analogue was present at a specific content ranging from less than 0.01 nmol·mg^{-1} in the individual with uninduced characteristics to nearly 0.30 nmol·mg^{-1} in

FIG. 3. Immunoblot of winter flounder hepatic microsomes. Lanes 1–3 contained 1.0, 3.0, and 5.0 pmol of purified scup cytochrome P450E, respectively. Lanes 4–11 contained 20 pmol of microsomal cytochrome P450 from fish 1–8, respectively, from New Bedford Harbor. Samples were blotted repeatedly with different amounts of microsomal P450 applied to ensure valid comparison of the relative amounts of cross-reacting protein.

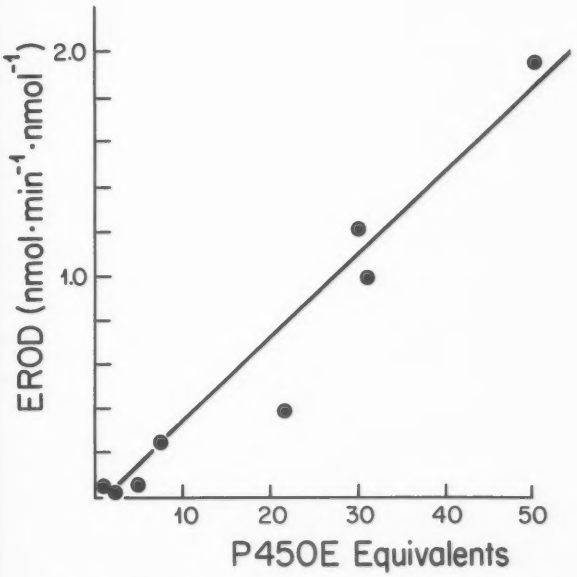

FIG. 4. Relationship between EROD activity and the relative amount of immunoreactive protein detected by MAb 1-12-3 in winter flounder from Buzzards Bay. Values on the *x*-axis are percent of microsomal P450 represented by the P450E equivalents, assuming similar affinity of MAb 1-12-3 for this antigen as for authentic P450E. The correlation coefficient (r^2) here was 0.94. Correlation between AHH activity and P450E (not shown) had an r^2 of 0.92.

the individual with the highest EROD activity. These values represent about 2 and 50% of the total microsomal P450, respectively.

Discussion

The results of this study show that hepatic microsomes of winter flounder from several sites in Massachusetts coastal waters have catalytic characteristics indicating PAH-type induction of cytochrome P450. The results establish that such catalytic evidence of induction is a common feature in winter flounder from diverse New England waters. Similar catalytic evidence for induced cytochrome P450 has been shown in winter flounder from coastal waters of Maine by Foureman et al. (1983).

In the present study, the induction indicated by catalytic data was unequivocally substantiated by immunoblot analysis. MAb 1-12-3 specifically recognized a single protein in the field sampled fish and in flounder treated with BNF. MAb 1-12-3 is highly specific for the BNF- or PAH-induced cytochrome P450E in scup, but also efficiently cross-reacts with the major BNF-induced form in other vertebrate species (Park et al. 1986; Kloepper-Sams et al. 1987). Based on these properties, we conclude that MAb 1-12-3 is recognizing a P450E homologue in winter flounder. Amounts of the flounder protein detected by MAb 1-12-3 correlated well with the levels of AHH and EROD activities, indicating that the flounder P450E counterpart is the AHH and EROD catalyst in this species. This is the first analysis of environmental induction demonstrating the use of heterologous monoclonal antibodies to induced teleost P450. Other such studies employing heterologous antibodies to induced teleost P450 used polyclonal antibodies to P450E (Stegeman et al. 1986) or to P450 LM_4 (Varanasi et al. 1986).

The correlation between the levels of EROD and AHH activity in winter flounder from Buzzards Bay was very strong ($r^2 = 0.98$), consistent with the suggestion that a single catalyst is involved. However, Foureman et al. (1983) saw less correlation between these activities in a larger group of winter flounder from Maine ($r^2 = 0.67$). In the present study, we also found little correlation between EROD and AHH activities in the winter flounder from Nantucket Sound. Discrepancies have also been noted between levels of these two activities in other studies (James and Bend 1980; Stegeman et al. 1981). Yet, both EROD and AHH activities are induced by PAH in fish. It could be that a range of activities as great as that seen in the Buzzards Bay fish, including a balance in the numbers of individuals that are slightly or not induced and those more highly induced, is necessary for such correlations to be strongly evident. Disparity between levels of EROD and AHH activities might also reflect different interactions of these substrates with one catalyst, or the presence of distinct catalysts for these activities. Studies are needed to clarify the relationships between levels of specific induced protein(s) and the different

catalytic functions of such proteins in order to accurately interpret results of environmental analysis based on these properties. Analysis with monoclonal antibodies directed toward different epitopes of induced proteins (Kloepper-Sams et al. 1987) may prove useful in this regard.

Origin of Induction

The sites here were chosen to represent different degrees of anthropogenic PAH and PCB contamination, based on content of these in sediment or resident bivalves (mussels) (Boehm et al. 1984; Farrington et al. 1982). Analysis of mussels from Boston Harbor, Plymouth Bay, Nantucket, and New Bedford Harbor (Farrington et al. 1982, 1983) showed relative PAH contents that were 12 000:2:1:8 and relative PCB content of 5:2:1:250, respectively. Although the high levels of activity in Boston Harbor fish correlate well with the very high PAH levels there, the levels of activity in Nantucket Sound or New Bedford Harbor fish do not correlate with the levels of PAH or PCBs at those sites.

Given the levels of PCB contamination in the New Bedford area, the presence of apparently uninduced animals in that vicinity is a condition we are as yet unable to explain. Several factors could contribute to this result. First, the precise chemical composition at the four sites is not known, and differences in the content or availability of specific inducers or other effectors of P450 could occur. Second, high levels of some compounds could cause a reduction in active P450 levels. In recent studies with trout (Melancon and Lech 1983), diminished monooxygenase activity was seen in animals treated with high levels of PCBs. We recently observed a similar phenomenon in scup (Stegeman et al., unpubl.), which we attribute to possible destruction of native P450 through suicide processing of substrates. The low levels of immunodetectable protein seen here are more consistent with a lack of induction than with any suicide or other inhibition by environmental compounds in vivo, degradation of activity during microsome preparation, or other factors that might influence the activity of induced proteins.

A third factor that could influence the distribution of "induction" is migration. A detailed study of winter flounder movements off Massachusetts (Howe and Coates 1975) showed that animals from Boston Harbor and Plymouth Bay migrate little. On the other hand, fish from Buzzards Bay tend to migrate outward during late spring and summer. It is thus improbable that Buzzards Bay fish had migrated from less polluted offshore waters, although migration to New Bedford Harbor from other regions of Buzzards Bay might occur (Howe and Coates 1975). Migration patterns might help explain high levels of induction found in fish from Nantucket Sound. Winter flounder from Buzzards Bay generally migrate to cooler offshore waters, including Nantucket Sound, in the summer, and some of the animals obtained there could have come from the more polluted Buzzards Bay area. However, those animals were collected in early October, long after such migration. If the induced winter flounder from Nantucket Sound were native to that region, it would suggest that some inducer(s), either natural or anthropogenic, are available there at biochemically significant levels. Additional studies of flounder from these waters will be required to correlate xenobiotic residues with induction. It may also be necessary to determine whether genetic or phenotypic differences (Howe and Coates 1975) could influence the expression of P450E homologues.

These questions notwithstanding, this study establishes that winter flounder from coastal Massachusetts are generally exposed to biochemically significant levels of some MC-type inducer of cytochrome P450. The consequences of this widespread induction, or the exposure it indicates, are not fully known. The activation of environmental carcinogens, e.g. B[a]P, to both cytotoxic and mutagenic metabolites by winter flounder hepatic preparations has been demonstrated (Stegeman et al. 1982). Given the relationships between P450E and the mammalian P450c, we propose that the P450E counterpart is responsible for this activation and that it is a required participant in initiation phases of tumorigenesis by some environmental chemicals in winter flounder. In addition to foreign compound activation, steroid metabolism is catalyzed by flounder liver cytochromes P450 (Snowberger and Stegeman 1987; Stegeman and Woodin 1984), an avenue whereby induction might affect reproductive processes.

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References

BINDER, R. L., AND J. J. STEGEMAN. 1980. Induction of aryl hydrocarbon hydroxylase activity in embryos of an estruarine fish. Biochem. Pharmacol. 29: 949–951.

BOEHM, P. D., W. STEINHAUER, AND J. BROWN. 1984. Organic pollutant biogeochemistry studies in the northeast U.S. marine environment. Part 1. The state of organic pollutant (PCB, PAH, coprostanol) contamination of the Boston Harbor – Massachusetts Bay – Cape Cod Bay system: sedlments and biota. NOAA Final Rep. Contract No. NA-83-FA-C-00022.

COUCH, J. A., AND J. C. HARSHBARGER. 1985. Effects of carcinogenic agents on aquatic animals: an environmental and experimental overview. Environ. Carcinog. Rev. 3: 63–105.

FARRINGTON, J. W., E. D. GOLDBERG, R. W. RISEBROUGH, J. H. MARTIN, AND V. T. BOWEN. 1983. U.S. "mussel watch" 1976–1978: an overview of the trace-metal, DDE, PCB, hydrocarbon, and artificial radionuclide data. Environ. Sci. Technol. 17: 490–496.

FARRINGTON, J. W., R. W. RISEBROUGH, P. L. PARKER, A. C. DAVIS, B. DE LAPPE, J. K. WINTERS, D. BOATWRIGHT, AND N. M. FREW. 1982. Hydrocarbons, polychlorinated biphenyls, and DDE in mussels and oysters from the U.S. coast 1976–1978 — the mussel watch. Woods Hole Oceanogr. Inst. Tech. Rep. WHOI-82-42.

FOUREMAN, G. L., N. B. WHITE, JR., AND J. R. BEND. 1983. Biochemical evidence that winter flounder (*Pseudopleuronectes americanus*) have induced hepatic cytochrome P450-dependent monooxygenase activities. Can. J. Fish. Aquat. Sci. 40: 854–865.

HOWE, A. B., AND P. G. COATES. 1975. Winter flounder movements, growth, and mortality off Massachusetts. Trans. Am. Fish. Soc. 1: 13–29.

JAMES, M. O., AND J. R. BEND. 1980. Polycyclic aromatic hydrocarbon induction of cytochrome P-450-dependent mixed-function oxidases in marine fish. Toxicol. Appl. Pharmacol. 54: 117–133.

JERINA, D. M., H. YAGI, D. R. THAKKER, J. M. SAYER, P. J. VAN BLADEREN, R. E. LEHR, D. L. WHALEN, W. LEVIN, R. L. CHANG, A. W. WOOD, AND A. H. CONNEY. 1984. Identification of the ultimate carcinogenic metabolites of the polycyclic aromatic hydrocarbons: Bay-region (*R*,*S*)-diol-(*S*,*R*)-epoxides, p. 257–266. *In* J. Caldwell and G. D. Paulson [ed.] Foreign compound metabolism. Taylor and Francis, London.

KLOEPPER-SAMS, P. K., S. S. PARK, H. V. GELBOIN, AND J. J. STEGEMAN. 1987. Specificity and cross-reactivity of monoclonal and polyclonal antibodies to cytochrome P-450E from the marine fish scup. Arch. Biochem. Biophys. 253: 268–278.

KLOTZ, A. V., J. J. STEGEMAN, AND C. WALSH. 1983. An aryl hydrocarbon hydroxylating cytochrome P-450 from the marine fish *Stenotomus chrysops*. Arch. Biochem. Biophys. 226: 578–592.

1984. An alternative 7-ethoxyresorufin *O*-deethylase activity assay: a continuous visible spectrophotometric method for measurement of cytochrome P-450 monooxygenase activity. Anal. Biochem. 140: 138–145.

LOWRY, O. H., N. S. ROSENBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MALINS, D. C., M. M. KRAHN, D. W. BROWN, L. D. RHODES, M. S. MYERS, B. B. MCCAIN, AND S-L. CHAN. 1985. Toxic chemicals in marine sediment and biota from Mukilteo, Washington: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulis*). J. Natl. Cancer Inst. 74: 487–494.

MELANCON, M. J., AND J. J. LECH. 1983. Dose-effect relationship for induction of hepatic monooxygenase activity in rainbow trout and carp by Aroclor 1254. Aquat. Toxicol. 4: 51–61.

MURCHELANO, R. A., AND R. E. WOLKE. 1985. Epizootic carcinoma in the winter flounder, *Pseudopleuronectes americanus*. Science (Wash., DC) 228: 587–589.

NEBERT, D. W., M. ADESNIK, M. J. COON, R. W. ESTRABROOK, F. J. GONZALEZ, F. P. GUENGERICH, I. C. GONSALUS, E. F. JOHNSON, B. KEMPER, W. LEVIN, I. R. PHILLIPS, R. SATO, AND M. R. WATERMAN. 1987. The P450 gene superfamily. Recomended nomenclature. DNA 6: 1–11.

PARK, S. S., H. MILLER, A. V. KLOTZ, P. J. KLOEPPER-SAMS, J. J. STEGEMAN, AND H. V. GELBOIN. 1986. Monoclonal antibodies against cytochrome P-450E of the marine teleost *Stenotomus chrysops* (scup). Arch. Biochem. Biophys. 249: 339–350.

RYAN, D. E., P. E. THOMAS, D. KORZENIOWSKI, AND W. LEVIN. 1979. Separation and characterization of highly purified forms of liver-microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. J. Biol. Chem. 254: 1365–1374.

SNOWBERGER, E. A., AND J. J. STEGEMAN. 1987. Patterns and regulation of estradiol metabolism by hepatic microsomes from two species of teleost fish. Gen. Comp. Endocrinol. 66. (In press)

STEGEMAN, J. J. 1987. Monooxygenase systems in marine fish. *In* C. S. Giam and L. Ray [ed.] Strategies in marine pollution monitoring. CRC Press, West Palm Beach, FL. (In press)

STEGEMAN, J. J., R. L. BINDER, AND A. ORRLN. 1979. Hepatic and extrahepatic microsomal electron transport components and mixed-function oxygenases in the marine fish *Stenotomus versicolor*. Biochem. Pharmacol. 28: 3431–3439.

STEGEMAN, J. J., AND P. J. KLOEPPER-SAMS. 1987. Cytochrome P-450 isozymes and monooxygenase activity in aquatic animals. Environ. Health Perspect. (In press)

STEGEMAN, J. J., P. J. KLOEPPER-SAMS, AND J. W. FARRINGTON. 1986. Monooxygenase induction and chlorobiphenyls in the deep-sea fish *Coryphaenoides armatus*. Science (Wash., DC) 231: 1287–1289.

STEGEMAN, J. J., A. V. KLOTZ, B. R. WOODIN, AND A. M. PAJOR. 1981. Induction of hepatic cytochrome P-450 in fish and the indication of environmental induction in scup. Aquat. Toxicol. 1: 197–212.

STEGEMAN, J. J., T. R. SKOPEK, AND W. G. THILLY. 1982. Bioactivation of polynuclear aromatic hydrocarbons to cytotoxic and mutagenic products by marine fish, p. 201–211. *In* N. L. Richards and B. L. Jackson [ed.] Carcinogenic polynuclear aromatic hydrocarbons in the marine environment. Proceeding of Conference, Pensacola FL, August 1978. U.S. E.P.A., Washington, DC.

STEGEMAN, J. J., AND B. R. WOODIN. 1984. Differential regulation of hepatic xenobiotic and steroid metabolism in marine teleost species. Mar. Environ. Res. 14: 422–425.

TOWBIN, H., T. STAEHELIN, AND J. GORDON. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets procedure and some applications. Proc. Natl. Acad. Sci. USA 76: 4350–4354.

VAN CANTFORT, J., J. DEGRAEVE, AND J. E. GIELEN. 1977. Radioactive assay for aryl hydrocarbon hydroxylase: improved method and biological importance. Biochem. Biophys. Res. Comm. 79: 505–512.

VARANASI, U., J. E. STEIN, D. E. WILLIAMS, AND D. R. BUHLER. 1986. Hepatic cytochrome P-450 isozymes and aryl hydrocarbon hydroxylase in English sole (*Parophrys vetulus*). Biochem. Pharmacol. 35: 2967–2971.

WILLIAMS, D. E., AND D. R. BUHLER. 1984. Benzo[a]pyrene-hydroxylase catalysed by purified isozymes of cytochrome P-450 from β-naphthoflavone-fed rainbow trout. Biochem. Pharmacol. 33: 3743–3753.

Comparison of Six Models for Nutrient Limitations on Phytoplankton Growth

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Morrison, K. A., N. Thérien, and B. Marcos. 1987. Comparison of six models for nutrient limitations on phytoplankton growth. Can. J. Fish. Aquat. Sci. 44: 1278–1288.

Equations and responses of six formulations for expressing nutrient limitation on phytoplankton growth are examined, including a scenario of no limitation. The Monod and internal storage formulations are representative of current applications, while the others have not been applied previously to phytoplankton. The general forms of growth responses under laboratory-type conditions are compared. The Contois formulation is found to be very flexible and is capable of describing the Monod and logistic formulations purely through selected parameter values. It can also generate a type of response not given by the other formulae. The different expressions are calibrated in a model applied to one station in the LG-2 reservoir. Validation is attempted using data from other stations. The Contois and logistic formulations gave the best results, but failures of the other formulations may be due in part to unavailability of much of the nutrients due to such things as complexing, while available data was only on total nutrients. Our results suggest that the Contois formulation is a valid alternative for representing nutrient limitation and may be robust in situations where data may be inadequate for the more rigourous internal storage formulations.

Les auteurs examinent six équations exprimant la limitation imposée par les bioéléments à la croissance du phytoplancton y compris l'absence de limitation. Les formules de Monod et d'entreposage interne représentent les applications actuelles tandis que les autres n'avaient pas encore été appliquées au phytoplancton. Les auteurs comparent les formes générales de la croissance dans des conditions expérimentales. La formule de Contois est très flexible et peut décrire les formules de Monod et logistique par la simple application de paramètres choisis. Elle peut aussi donner un genre de réaction qui n'est pas générée par les autres formules. Les différentes expressions sont étalonnées dans un modèle appliqué à une station du réservoir LG-2. On effectue aussi une tentative de validation à l'aide de données recueillies à d'autres stations. Les formules de Contois et logistique ont donné les meilleurs résultats mais l'échec des autres formules peut être dû en partie à l'indisponibilité de la plus grande partie des bioéléments à cause de facteurs comme la complexation; par contre, les données disponibles ne concernaient que la teneur totale en bioéléments. Les résultats portent à croire que la formule de Contois peut exprimer la limitation imposée par les bioéléments et peut s'appliquer dans des situations où les données sont inadéquates pour l'analyse à l'aide de formules d'entreposage internes plus précises.

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Simulation models are currently used to a limited extent for environmental impact assessments, and their use is bound to grow in the near future (Beanlands and Duinker 1983). Seldom is any "off-the-shelf" model immediately applicable to all situations. Instead, models must be adapted to the peculiarities of a given situation (e.g. species composition), and one of these adaptations is the recalibration of certain model parameters to fit baseline preimpact data. The complexity of calibration increases greatly with the number of parameters to be adjusted. Thus, given a choice between two models that can represent the same processes equally well, the model with fewer parameters is easier to apply as well as to understand.

Aquatic systems are frequently impacted in development projects, especially via increased nutrient loadings. The nutrients may come from wastewater release, increased soil erosion, or plant and soil decomposition after innundation. Since nutrients have a marked effect on aquatic primary production, they are usually included in aquatic ecological models. The mathematical functions used to account for the nutrient effects differ, sometimes substantially, from one model to another. The major differences are related to whether the limitations of nutrients on growth are due to external or internal nutrient concentrations, and these two points of view are typified by the Monod (or Michaelis–Menton) and internal storage formulations.

These two formulations represent two extremes for the number of parameters required to express the limitation of a nutrient. The Monod formulation requires only one parameter per nutrient, while the internal storage models require four or more (Bierman 1981). In this work we examine the actual limitations to growth imposed by these formulations and assess the Contois (1959) formulation, which has the same complexity as the Monod expression but much greater flexibility. For the sake of comparison we also examine the logistic formulation, a modification of the storage formulation, and a model imposing no nutrient limitation. The modification to the storage formulation is the use of a Contois-type term in the uptake expression rather than a Monod-type term and was made because Williams

(1973) and Li (1983) observed that the half-saturation constant for uptake seemed to vary with biomass. The scenario of no nutrient effects is included as a null hypothesis. We consider the effects of total phosphorus and total nitrogen. Each formulation has been fit (optimized) to the data coming from one sampling station located in a reservoir in northern Québec. Only coefficients pertaining to phytoplankton have been optimized, and densities of zooplankton as grazers have been fed in as input data. After this calibration, each formulation was applied to data spanning a longer period from this station and from two others. Some of the faults of the formulations are highlighted by this examination, at least from a pragmatic point of view.

Model Equations

In simulation models the dynamic variables under examination are called state variables and each is represented by a differential equation. Phytoplankton may be grouped together as one state variable or may be divided into several state variables on the basis of major taxa (e.g. diatoms, desmids, cyanobacteria, etc.).

A typical differential equation for phytoplankton is

$$(1)\quad d\frac{\text{PHYTO}}{dt} = \text{GROWTH} - \text{RESP} - \text{SED} - \text{PREDZ1} - \text{PREDZ2}$$

where GROWTH = primary production rate ($mg \cdot L^{-1} \cdot d^{-1}$), given by

$$(2)\quad \text{GROWTH} = \text{GRMAX} \times f(\text{LIGHT}) \times f(\text{NUTR}) \times f(\text{TEMP}) \times \text{PHYTO}$$

where GRMAX = maximum relative growth rate (d^{-1}), f(LIGHT) = limitation due to light intensity (dimensionless), f(NUTR) = limitation due to nutrients (dimensionless), given by

$$(3)\quad f(\text{NUTR}) = \text{MINIMUM (LIM(PHOS), LIM(NITR))},$$

f(TEMP) = limitation due to temperature (dimensionless), PHYTO = phytoplankton biomass concentration ($mg \cdot L^{-1}$), RESP = respiration rate ($mg \cdot L^{-1} \cdot d^{-1}$), given by

$$(4)\quad \text{RESP} = \text{RESPMAX} \times f(\text{TEMP}) \times \text{PHYTO}$$

where RESPMAX = maximum relative respiration rate (d^{-1}), SED = combined mortality−sedimentation term ($mg \cdot L^{-1} \cdot d^{-1}$), given by

$$(5)\quad \text{SED} = \text{SEDMAX} \times f(\text{VISC}) \times \text{PHYTO}$$

where SEDMAX = maximum relative sedimentation rate (d^{-1}) and f(VISC) = limitation of water viscosity on sedimentation rate (dimensionless), given by

$$(6)\quad f(\text{VISC}) = 0.56 + 0.022(^{\circ}C^{-1}) \times \text{TEMP } (^{\circ}C),$$

PREDZ1 = grazing on phytoplankton by filter-feeding zooplankton ($mg \cdot L^{-1} \cdot d^{-1}$), given by

$$(7)\quad \text{PREDZ1} = \text{PREDMAX1} \times f(\text{TEMP}) \times \frac{\text{PHYTO}}{\text{PHYTO} + \text{PRS1} \times \text{SESTON} + \text{KPZ1}} \times \text{Z001}$$

where PREDMAX1 = maximum overall rate of ingestion of filter-feeders (mg phytoplankton · mg filter-feeders$^{-1} \cdot d^{-1}$), PRS1 = relative preference for SESTON by filter-feeders (mg phytoplankton · mg seston^{-1}), KPZ1 = half-saturation constant for ingestion ($mg \cdot L^{-1}$), and Z001 = biomass concentration of filter-feeders ($mg \cdot L^{-1}$), and PREDZ2 = grazing on phytoplankton by raptorial zooplankton ($mg \cdot L^{-1} \cdot d^{-1}$), given by

$$(8)\quad \text{PREDZ2} = \text{PREDMAX2} \times f(\text{TEMP}) \times \frac{\text{PHYTO}}{\text{PHYTO} + \text{PRS2} \times \text{SESTON} + \text{KPZ2} \times \text{Z002}} \times \text{Z002}$$

where PREDMAX2 = maximum relative rate of ingestion of raptors (mg phytoplankton · mg raptors$^{-1} \cdot d^{-1}$), PRS2 = relative preference for SESTON by raptors (mg phytoplankton · mg seston^{-1}), KPZ2 = Contois-type half saturation constant for ingestion (mg phytoplankton · mg raptors^{-1}), and Z002 = biomass concentration of raptors ($mg \cdot L^{-1}$). Readers interested in details on the rest of the model are referred to Thérien et al. (1982).

It is the terms LIM(PHOS) and LIM(NITR) that are calculated according to the different formulations as functions of the specific nutrient concentration considered by the formulation.

For the following discussion of the formulations, we will consider a generic nutrient NUTR. It is useful to define some of the terms for nutrient concentrations NUTR: NUTT = total usable concentration of nutrient in question ($mg \cdot L^{-1}$), NUTE = external usable concentration of nutrient ($mg \cdot L^{-1}$), NUTI = internal concentration of nutrient ($mg \cdot L^{-1}$), and NUTIR = internal nutrient concentration per unit biomass concentration (mg nutrient · mg biomass^{-1}). These definitions lead to the following expressions:

$$(9)\quad \text{NUTT} = \text{NUTE} + \text{NUTI}$$

$$(10)\quad \text{NUTI} = \text{NUTIR} \times \text{PHYTO}$$

$$(11)\quad \text{NUTE} = \text{NUTT} - \text{NUTIR} \times \text{PHYTO}.$$

Other expressions can be derived from these.

Formulations and Responses

The effects of nutrient concentrations on relative growth rate are best compared for the different formulations using a laboratory experiment type condition: the total concentration of nutrient (internal + external) is fixed. We shall compare the responses of the formulations over the range of biomass from 0 to the carrying capacity, and the response curves are standardized to have the same range (Fig. 1). Carrying capacity is explained in the next section. At each point on the curves, the total nutrient is allocated to internal and external using expression (11), or for the storage formulations, differencing of expression (9).

Monod Formulation

The Monod kinetics formulation (Monod 1942, 1949) is extremely simple and was the first expression used in ecological models. It has the form

$$(12)\quad \text{LIM(NUTE)} = \text{NUTE} / (\text{NUTE} + \text{K1})$$

where LIM(NUTE) = limitation on growth due to NUTE and K1 = half-saturation constant for limitation on growth due to NUTE ($mg \cdot L^{-1}$).

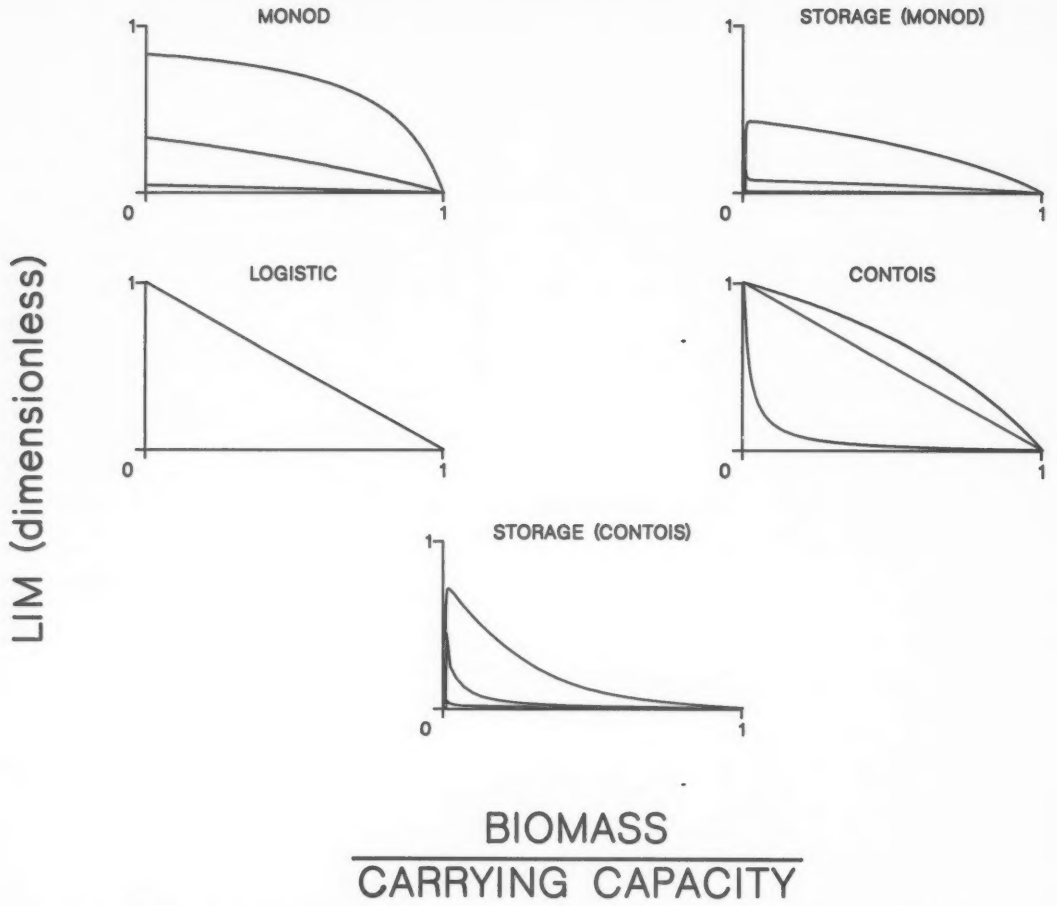

FIG. 1. Typical response curves for the formulations over the range of biomass from zero to the carrying capacity.

Only one constant need be estimated to use this formulation. It is still a very popular approach, as shown by Swartzman and Bentley (1979), who found it in 8 of 12 models reviewed. There is in fact an implicit additional constant for this formulation: the relative internal concentration of the nutrient, NUTIR, also called the yield coefficient. This parameter corresponds to the constant stoichiometry of the cells, and Monod (1942) defined NUTE using the expression given in equation (11). Thus, to use the Monod formulation with a measure of total nutrient concentration, the formulation becomes

$$(13)\quad \mathrm{LIM(NUTE)} = \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} + \mathrm{K1})}.$$

The curve with a very low half-saturation constant has convex form, as should be expected since a low constant means high sensitivity to low nutrient concentrations. The progressively higher half-saturation constants result in curves progressively less convex, and with progressively lower intersects on the ordinate.

The adequacy of the Monod formulation has been called into question for phosphorus and nitrogen (Bierman 1981; Collins 1980; Jorgensen 1980), with internal storage formulations being preferred.

Internal Storage Formulations

Internal storage models consider explicitly internal nutrient as an additional state variable for each nutrient being examined, requiring an additional differential equation:

$$(14)\quad d\mathrm{NUTI}/dt = \mathrm{UPTAKE} - \mathrm{LOSSN}$$

where UPTAKE = rate of uptake of NUTE into NUTI ($\mathrm{mg \cdot L^{-1} \cdot d^{-1}}$) and LOSSN: rate of loss of NUTI due to mortality, predation, etc. ($\mathrm{mg \cdot L^{-1} \cdot d^{-1}}$). The rationale for these formulations comes from work by Rhee (1973, 1974) and Droop (1968, 1973), among others.

There are many different formulations for internal storage models. We examine the formulation used by Jorgensen (1980). The first expression is for uptake and, modified from Jorgensen (1980), is

$$(15)\quad \mathrm{UPTAKE} = \mathrm{UPMAX} \times \mathrm{PHYTO} \times \frac{(\mathrm{NUTIRMX} \times \mathrm{PHYTO} - \mathrm{NUTI})}{(\mathrm{PHYTO} \times (\mathrm{NUTIRMX} - \mathrm{NUTIRMN}))} \times \frac{\mathrm{NUTE}}{\mathrm{NUTE} + \mathrm{K1}}$$

where UPMAX = maximum relative uptake rate per unit biomass concentration (d^{-1}), NUTIRMX = maximum relative internal nutrient concentration per unit biomass concentration, and NUTIRMN = minimum relative internal nutrient concentration per unit biomass concentration. To use this expression with only a measure of total nutrient, one would find NUTE by difference using expression (9).

The limitation on growth is given by the Droop cell quota model (Droop 1968):

$$\text{LIM(NUTI)} = (\text{NUTI} - \text{NUTIRMN} \times \text{PHYTO})/\text{NUTI}. \quad (16)$$

As can be seen, for each nutrient, three parameters (UPMAX, NUTIRMX, and NUTIRMN) are required in addition to the half-saturation constant K1. Other internal storage formulations can require even more parameters (e.g. Collins 1980).

For the internal storage formulation, we shall use the formulation of Jorgensen (1980) as already presented. Since this is a dynamic formulation, the actual responses will depend on a number of factors. We have therefore limited our examination to some representative responses by using three different values for maximum uptake and have integrated the equations for nutrients and biomass at the same time.

Notice that the three curves start at LIM = 0 and increase to a maximum value, from which the curves descend. This is due to us starting the integration with a very low biomass density considered to have minimum internal nutrients. The curves are similar to the normal Monod curves, although lower due to choices of parameter values.

Logistic Formulation

The typical logistic expression is

$$\text{LIM} = (C - N)/C = 1 - (N/C) \quad (17)$$

where C = carrying capacity and N = population. For the sake of arguement, we will take population as being biomass and carrying capacity as being a function of total nutrients (i.e. C = K3 × NUTT), since it can potentially be assimilated. Thus the limitation becomes

$$\text{LIM(NUTE)} = (\text{K3} \times \text{NUTT} - \text{PHYTO})/(\text{K3} \times \text{NUTT}) = 1 - (\text{PHYTO}/\text{K3} \times \text{NUTT}) \quad (18)$$

where K3 = relative carrying capacity per unit nutrient concentration (mg biomass · mg nutrient^{-1}). This formulation requires only one parameter, K3. This parameter is in fact the reciprocal of NUTIR, and the formula may be rewritten

$$\text{LIM(NUTE)} = (\text{NUTT} - \text{PHYTO} \times \text{NUTIR})/\text{NUTT} = \text{NUTE}/\text{NUTT} = 1 - (\text{PHYTO} \times \text{NUTIR})/\text{NUTT}. \quad (19)$$

The logistic formulation uses only one value for the relative carrying capacity, since it has been standardized with the other formulations for the range of biomass/carrying capacity. The logistic formulation results in a constant negative slope with increasing relative biomass density. Note the intercept with the ordinate.

Monod (1942) discussed the logistic formulation, but we have not found an example of its application to nutrients in ecological models.

Contois Formulation

The Contois formulation came from examinations of continuous cultures, although its applicability to batch systems was noted (Contois 1959). This formulation has one difference from the Monod expression: the half-saturation constant is multiplied by biomass. The formulation is therefore

$$\text{LIM(NUTE)} = \text{NUTE}/(\text{NUTE} + \text{K2} \times \text{PHYTO}). \quad (20)$$

An alternative way to write the formulation is

$$\text{LIM(NUTE)} = (\text{NUTE}/\text{PHYTO})/(\text{NUTE}/\text{PHYTO} + \text{K2}). \quad (21)$$

Thus, K2 in this formulation is a dimensionless half-saturation constant expressing the effect of the amount of available nutrient per unit biomass on relative growth, much the same as the expression used by Collins (1980) for limitation by internal nutrient. As with the Monod expression, NUTIR is an implicit parameter for the expression to be used with total nutrient concentrations, and the formulation may be rewritten

$$\text{LIM(NUTE)} = \frac{(\text{NUTT} - \text{NUTIR} \times \text{PHYTO})}{(\text{NUTT} - \text{NUTIR} \times \text{PHYTO} + \text{K2} \times \text{PHYTO})}. \quad (22)$$

The three values used for the half-saturation constants in the Contois formulation correspond to K2 less than, equal to, and greater than NUTIR. These three values translate into convex, linear, and concave curves, respectively. The linear curve is identical to the logistic formulation.

Modified Internal Storage Formulation

The modification we try for the internal storage formulation is simply to replace the last term in equation (15), a Monod expression, with the corresponding Contois expression. Thus, instead of equation (15), one has

$$\text{UPTAKE} = \text{UPMAX} \times \text{PHYTO} \times \frac{(\text{NUTIRMX} \times \text{PHYTO} - \text{NUTI})}{(\text{PHYTO} \times (\text{NUTIRMX} - \text{NUTIRMN}))} \times \frac{\text{NUTE}}{\text{NUTE} + \text{K2} \times \text{PHYTO}}. \quad (23)$$

The same general comments as for the other storage formulation apply. One major difference is that these curves are concave because the other parameters are not varied.

Mechanisms of Action and Carrying Capacity

Uptake is accounted for explicitly in the internal storage formulations and implictly in the others. All formulations have feedback mechanisms. For example, an increase in total nutrients is always translated into an increase in the LIM term, indirectly via uptake in the internal storage models and directly for the others. This translates into an increased growth rate, with a resulting increase in biomass. The increased biomass assimilates more nutrients, reducing the amount available and thus decreasing growth, again indirectly in the internal storage formulations. All formulations include a global carrying capacity at which the growth rate is reduced to 0. This carrying capacity is a function of total nutrient concentration and is the point at which all nutrients have been assimilated. For the

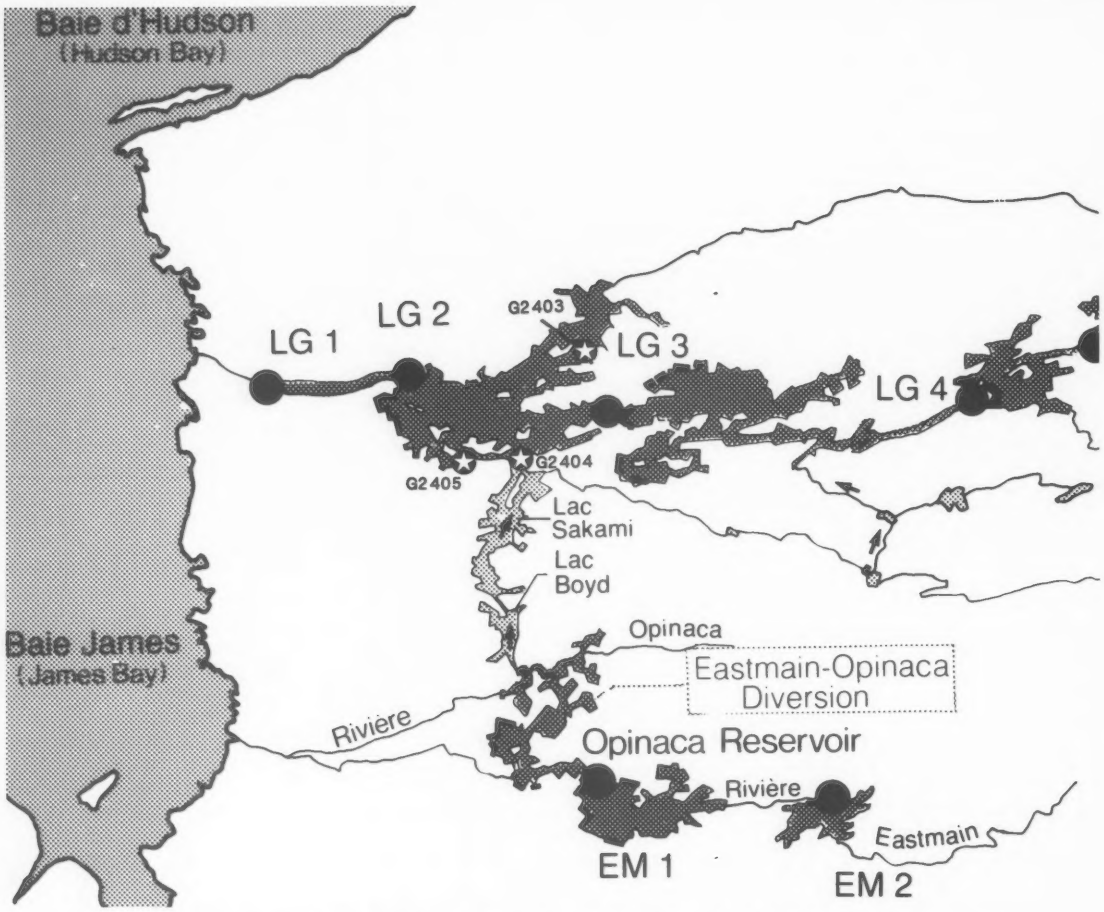

FIG. 2. La Grande Hydro-electric Complex, showing the location of the sampling stations within the LG 2 reservoir.

Monod, logistic, and Contois formulations this carrying capacity is

(24) $CC = NUTT/NUTIR.$

For the internal storage formulations it is

(25) $CC = NUTT/NUTIRMN.$

These two equations are in fact the same because the constant-stoichiometric formulations take NUTIR to be the same as NUTIRMN. The major differences among the formulations come in the actual values of the limitations over the range of biomass from 0 to the carrying capacity.

Experimental Data and Optimization

The sampling stations are located within the LG 2 reservoir of the La Grande complex near James Bay in Québec (Fig. 2). The three stations were in different lakes prior to reservoir inundation, and this is reflected in their somewhat different concentrations of total phosphorus and nitrogen (Fig. 3).

Data on nutrient concentration, temperature, zooplankton biomass, and chlorophyll *a* were provided by the Environment Directorate of La Société d'Energie de la Baie James (SEBJ). Chlorophyll *a* values were converted to phytoplankton biomass using a factor of 100 (Ahlgren 1970). Zooplankton counts were converted to biomass on a per-species basis using mean measured weights. Linear interpolation was used to generate values of the input variables (nutrients, temperature, and zooplankton) between sampling dates. Information on sampling methods can be found in Thérien et al. (1982). Nutrients were evaluated using an auto-analyser.

For each formulation, the maximum growth, respiration, and sedimentation rates for phytoplankton were among the parameters calibrated. For the formulation with no limitation, these were the only parameters calibrated. For the Monod, Contois, and logistic formulations, one parameter was optimized for the limitation expression of each nutrient. For both internal storage formulations, two parameters (maximum uptake rate and the half-saturation constant for uptake) were optimized for each nutrient. In all cases, constants associated with grazing were fixed at the same values.

Optimization was done using the Marquardt method (Marquardt 1963) as applied to differential equations (Bard 1970). The optimization was carried out using the data from station G2

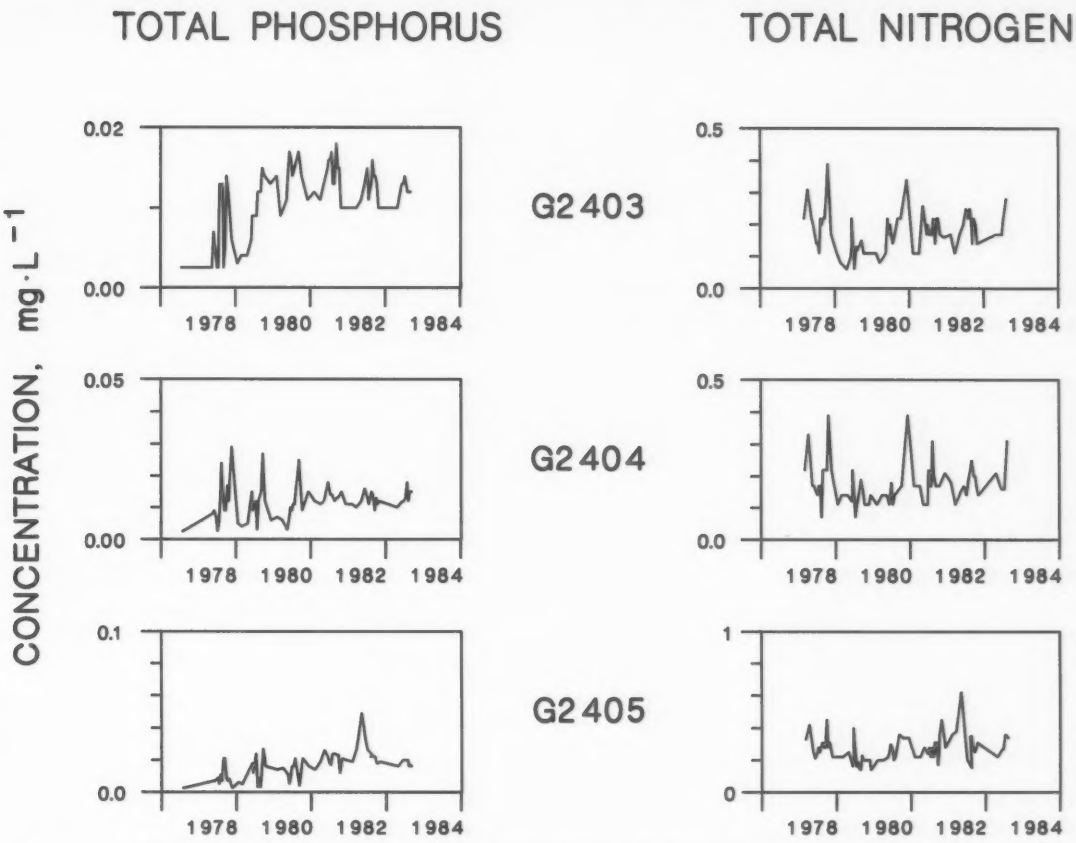

FIG. 3. Observations of total phosphorus and total nitrogen at the three sampling stations over time.

TABLE 1. Parameter values as calculated by the optimization procedure and subsequently applied to the data from the three stations. For all formulations, minimum internal relative concentrations of phosphorus and nitrogen were taken to be 0.001 and 0.015, respectively, and maximum internal relative concentrations for the storage formulations were 0.013 and 0.1, respectively. Parameter units are defined in the text; NA = not applicable.

Formulation	GRMAX	RESPMAX	SEDMAX	K1, K2, or K3 (phosphorus)	K1, K2, or K3 (nitrogen)	UPMAX (phosphorus)	UPMAX (nitrogen)
Monod	1.997	0.0686	0.0321	0.00344	0.0271	NA	NA
Contois	2.682	0.0802	0.0249	0.0475	0.6794	NA	NA
Logistic	2.803	0.0947	0.0302	0.0275	0.2658	NA	NA
No limitation	0.664	0.0623	0.0010	NA	NA	NA	NA
Storage (Monod)	1.281	0.0878	0.0119	0.0611	0.9957	0.00124	0.1375
Storage (Contois)	1.900	0.0700	0.0350	0.2220	0.1548	0.00092	0.0146

403 for the period from midsummer 1978 to spring 1981. The choice of G2 403 over the other stations was entirely arbitrary. The parameter values are shown in Table 1.

Results and Discussion

In the optimizations for station G2 403 (Fig. 4), the Monod formulation appears to give the best fit, although in 1982, phytoplankton is severely reduced and goes to extinction. The Contois and logistic formulations fit the data well over the period of the optimization, but give short-lived peaks in 1981 and 1982. The no-limitation, Monod storage, and Contois storage models all predict almost extinction after 2 yr.

Applied to station G2 404 (Fig. 5), only the Monod storage formulation predicts extinction, while the Contois storage formulation shows a gradually decreasing density. The Monod formulation strongly overpredicts biomass densities. The no-limitation overpredicts completely. The Contois and logistic formulations provide reasonably good results. All formulations overpredict for the summer of 1979, the period of reservoir filling.

Applied to station G2 405 (Fig. 6), none of the formulations

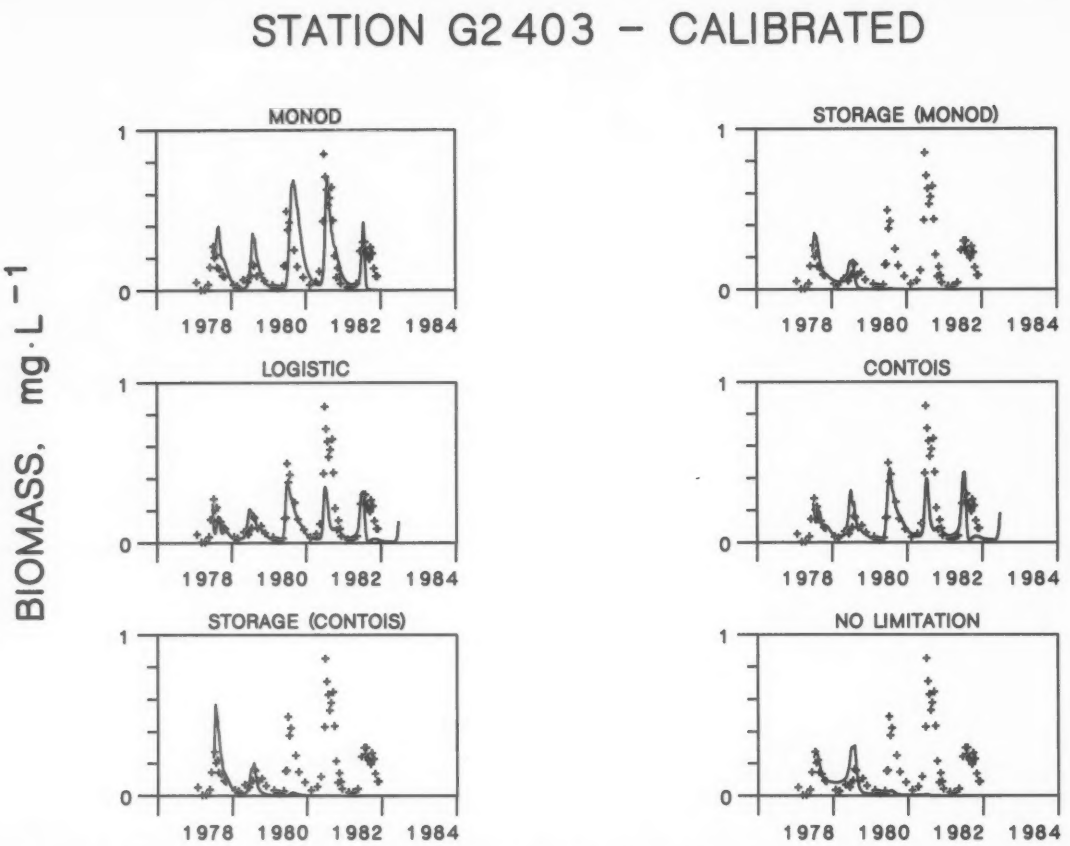

FIG. 4. Observed (+) and predicted (—) values for phytoplankton density at station G2 403. The period of simulation is from 1978 to 1983, but the model fits were optimized over the first 3 yr.

gives what could be called a good fit, but the Contois and logistic formulations give the best results. The Monod formulation overpredicts for 2 yr, followed by extinction. The no-limitation and Monod storage approaches both predict immediate extinction, while the Contois storage formulation predicts extinction after a couple of years.

Of the six different formulations, the Contois and logistic formulations provided the most consistent results. The Monod formulation provided a good fit only for G2 403 for the calibration process. The other two stations were somewhat richer in nutrients, and overestimation resulted. The model using no limitation did not give acceptable results for any of the applications, highlighting how important nutrient limitations are. The storage formulations also did not give acceptable results.

One important point should be noted for the two storage formulations. During the optimization procedures, we found that local minima presented problems for both of these formulations, and a large number of trials with different initial values for the coefficients were needed. We are thus not certain that we found the "best" combinations of coefficient values for these formulations. Such calibration problems would probably not be acceptable for an impact assessment project.

Our approach to these simulations was somewhat rigid because we removed the phytoplankton from the predator–prey system by using zooplankton concentrations as input data. Thus, there was no feedback among state variables, and extinction of phytoplankton was possible when growth was particularly low.

One irritant pertaining to the logistic formulation is that the coefficients for both nutrients should be close to the minimum internal relative concentrations, but instead are an order of magnitude larger. This could be a reflection of unavailability of a large part of the nutrients due to, for example, phosphorus complexing with iron. This could explain at least some of the problems with the storage and Monod formulations. With the coefficient being an order of magnitude too large, the carrying capacity in this formulation is an order of magnitude smaller than for the others. For the Contois formulation, the values of K2 cause an extremely concave response (Fig. 1), so that most growth is occurring far below the carrying capacity. The other formulations are not able to duplicate such behaviour. If we had had data on usable rather than total nutrients, it is quite possible that we would have had better success with the other three formulations, particularly the storage formulations.

Our evaluation has been on a pragmatic level. In a proper scientific study of plankton dynamics, one would certainly not deal with such crude entities as total phosphorus or total nitrogen. In situations of environmental impact assessment, however, there are frequently only such crude measurements available. For such situations, our results suggest that for a model

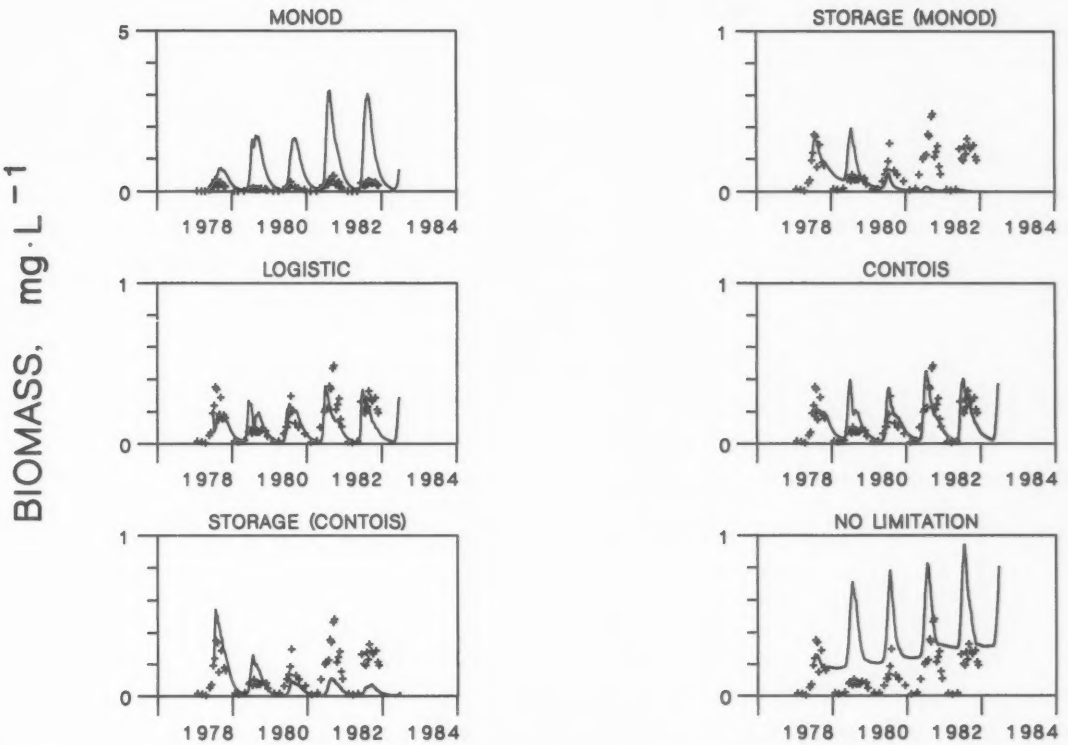

FIG. 5. Observed (+) and predicted (—) values for phytoplankton density at station G2 404. The parameter values used for the models were those fit to station G2 403, and no recalibration was done. Note that the scale for the ordinate is different for the Monod results due to overestimation.

of plankton dynamics, the Contois or logistic formulations may be the preferred approaches for representing nutrient limitations. The Monod formulation was not satisfactory. The two storage formulations should probably only be applied in situations where very specific and detailed data are available. The storage formulation incorporating the Contois limitation for uptake is possibly preferable to that using the Monod limitation, but we have not seen any applications to laboratory data. It would explain the differences of uptake noted by Tarapchak and Herche (1986) only if significant growth was occurring in their experiments.

The Contois formulation can generate a greater repertoire of response curves than the other formulations. Not only can it generate convex to linear responses, but it can also generate concave responses. The concave response was examined by Monod (1942) and was rejected as being impossible in closed homogeneous cultures. He noted, however, that such a curve could be found in nonhomogeneous systems or in systems where conditions were not held constant. These are precisely the conditions found in natural waters. Patchiness and gradients are constantly present, causing such systems to be nonhomogeneous, plus the systems are constantly undergoing change.

The Contois formulation is really a generalized form of the Monod formulation. The half-saturation constants have different meanings, but the two curves represent the same relative limitation for batch cultures when the Contois function is convex. This can be shown by defining relative limitation as normalized to a range of 0–1 by

$$\text{LIMR} = \text{LIM}/\text{LIMMAX} \quad (26)$$

where LIMR = relative limitation and LIMMAX = maximum value for the limiting curve. In a batch experiment with a fixed amount of total nutrient NUTT, the maximum value for the relative limitation calculated by the Monod formulation occurs for 0 biomass and is less than 1, being

$$\text{LIMMAX} = \text{NUTT}/(\text{NUTT} + \text{K1}). \quad (27)$$

For the Contois formulation, at 0 biomass the maximum value is

$$\text{LIMMAX} = \text{NUTT}/(\text{NUTT} + \text{K2} \times 0) = \text{NUTT}/\text{NUTT} = 1. \quad (28)$$

If one wishes to normalize the Monod expression to the range 0–1, calling this normalized value LIMR, one only has to divide the limitation term by LIMMAX, and thus:

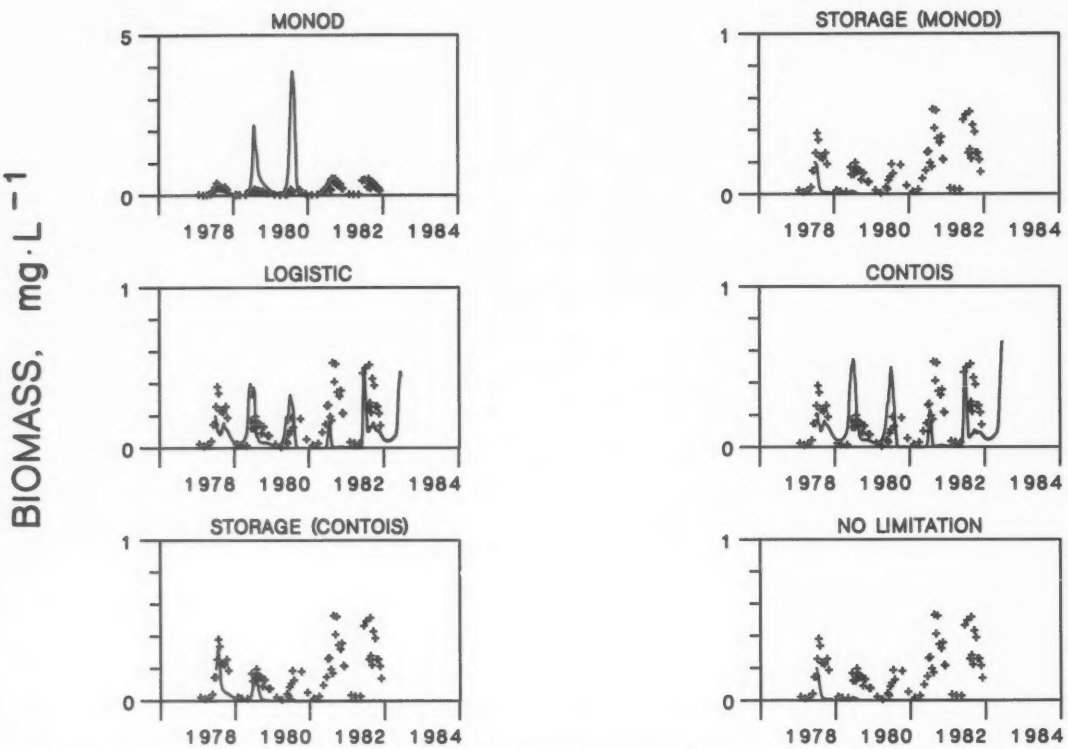

FIG. 6. Observed (+) and predicted (—) values for phytoplankton density at station G2 405. As for G2 404, parameter values for the models were not recalibrated. Note again that the scale of the ordinate for the Monod results is larger due to overestimation.

$$(29)\quad \mathrm{LIMR} = \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})/(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} + \mathrm{K1})}{(\mathrm{NUTT}/(\mathrm{NUTT} + \mathrm{K1}))}$$

$$= \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{\mathrm{NUTT} \times (\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} + \mathrm{K1})/(\mathrm{NUTT} + \mathrm{K1})}$$

$$= \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{\mathrm{NUTT} \times [(\mathrm{NUTT} + \mathrm{K1})/(\mathrm{NUTT} + \mathrm{K1}) - \mathrm{NUTIR} \times \mathrm{PHYTO}/(\mathrm{NUTT} + \mathrm{K1})]}$$

$$= \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{\mathrm{NUTT} \times [1 - \mathrm{NUTIR} \times \mathrm{PHYTO}/(\mathrm{NUTT} + \mathrm{K1})]}$$

$$= \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} \times (\mathrm{NUTT}/(\mathrm{NUTT} + \mathrm{K1}))}$$

$$= \frac{(\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO})}{\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} \times \left[1 - \dfrac{\mathrm{K1}}{(\mathrm{NUTT} + \mathrm{K1})}\right]}$$

$$= \frac{\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO}}{\mathrm{NUTT} - \mathrm{NUTIR} \times \mathrm{PHYTO} + \dfrac{\mathrm{NUTIR} \times \mathrm{K1}}{\mathrm{NUTT} + \mathrm{K1}} \times \mathrm{PHYTO}}$$

which is the same as a Contois expression with

$$(30)\quad \mathrm{K2} = \frac{\mathrm{NUTIR} \times \mathrm{K1}}{\mathrm{NUTT} + \mathrm{K1}}.$$

If one takes NUTIR as the minimum internal relative concentration, all terms in this expression are constant. Expression (30) can be rearranged to give

(31) $K1 = (K2 \times NUTT)/(NUTIR - K2)$.

Thus, for a laboratory experiment that starts with a fixed amount of total nutrient NUTT and that follows the subsequent growth of biomass, the Contois formulation can generate a limitation curve directly proportion to a Monod expression:

(32) $LIM(MONOD) = LIM(CONTOIS) \times NUTT/(NUTT + K1)$

and any estimated maximum relative rate would be inversely proportional in the same way. In a series of similar experiments with different amounts of total nutrient NUTT, the same value of K2 will equate to different values of K1 proportional to NUTT (as noted by Contois (1959)), suggesting higher sensitivity to nutrients at lower nutrient levels. However, in experiments with equal biomass and varying nutrients which are of such limited duration that significant growth does not occur, the two expressions will be indistinguishable because the term $K2 \times PHYTO$ will be effectively constant. In experiments with different densities of biomass (PHYTO), the same value of K2 will equate to different values of K1 proportional to PHYTO, and such variation has been found (Goldman 1977). Variation of K1 under these circumstances has also been noted in laboratory experiments on microbial uptake (Williams 1973; Li 1983), although variation is less marked for short-term experiments where growth is not significant. Therefore, in a dynamic open system the effect of the Contois formulation will differ substantially from that of the Monod expression, since both NUTT and PHYTO will vary with time. Auer et al. (1986) remarked that the Monod expression could not address non-constant nutrient concentrations.

The application of the Monod formulation to open dynamic systems is in contradiction with the discussion of Monod (1942). Most articles referring to this formulation cite Monod (1949), from which the reader is referred back to the earlier work for details. Confusion seems to have arisen because many researchers did not go back to the original reference to find the context of application of the formulation.

Even where the Monod formulation has been applied to laboratory cultures, one potential source of problems can be seen. Monod (1942) was in fact applying equation (13), while researchers tend to apply equation (12). There is a subtle difference between the two. Equation (13) states that there is a minimum amount of nutrient per unit biomass, and any nutrient in excess of this amount is available for growth, regardless of whether or not it is already absorbed into the cells. Equation (12) explicitly states that only nutrients external to the cells are available for growth. These two would only be equal if the actual internal nutrient concentration was strictly constant, which of course it is not. NUTE as defined by expression (11) should be considered as available nutrient rather than strictly external nutrient, and NUTIR in this expression should in fact be equal to the minimum internal nutrient NUTIRMN.

If one reexamines expression (21), one can see the nature of the action of the Contois formulation as compared with the Monod formulation. The Contois formulation, as the equivalent of a Monod expression for the ratio of available nutrient/biomass rather than available nutrient alone, is in fact accounting for competition for available resources. A possible mechanism for such a competitive effect is the excretion of inhibitory compounds, a well-known occurrence (Wetzel 1975). The concentration of such compounds will be at least partially a function of biomass. Therefore, for a fixed amount of available nutrient, one would expect relative growth (per unit biomass) to be somewhat lower when more biomass is present. Expression (20) has this type of behaviour, since the equivalent of the half-saturation constant in a Monod expression is a function of biomass, i.e. $K2 \times PHYTO$.

We are not the first to apply a Contois-type formula to an ecological model. MacCormick et al. (1972) and Jorgensen (1980) used it to express the effects of competition on grazing by zooplankton on phytoplankton, also considered important by Thérien et al. (1982). We have not encountered the application of this formulation to total or external nutrients anywhere in the literature, although Collins (1980) used a similar formula in place of the Droop cell quota model for limitation by internal nutrients.

While it is generally recognized that the biological detail of internal storage models is a fair approximation in the actual mechanisms controlling growth, appropriate data for validating and/or calibrating such a formulation are frequently available only in controlled laboratory experiments using batch cultures. It is not yet clear how applicable the current formulae are for open systems, but it must be noted that we have not examined all of the variations of internal storage models that have been proposed. If nutrient cycling was an important aspect of the model in use, a storage formulation would be necessary.

One potential difficulty with the two storage formulations is the difference in time scales between uptake, which in laboratory experiments has been observed on a scale of minutes, and growth which is on a scale of days. Since simulation of natural systems is always on a longer time scale, these formulations may continue to be difficult to apply. The effect of this discrepancy is entirely numerical. Di Toro (1980) recognized this problem and suggested a compromise formulation, but we have not seen others apply it.

That the data requirements are far less stringent for the Monod expression is without doubt a major reason favouring its use for natural systems. This is especially true, since the formulation can be applied to measurements of total nutrient, although an additional parameter is required. The Contois formulation has the same requirements as the Monod expression, but provides much more flexibility and seems more robust. Sensitivity analyses may be necessary to verify robustness. Whether or not fixed stoichiometry limits the usefulness of the Contois expression as much as the Monod expression can only be determined from further applications.

A point to note is that had the comparisons been based solely on 1 yr of data, the shortcomings of the formulations would not have been as clear. Simons and Lam (1980) noted difficulties with calibration based only on a 1-yr period. Thus, 1 yr of data may not be sufficient for model calibration and/or validation, although this is a frequent practice (e.g. Collins 1980).

Acknowledgements

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References

AHLGREN, G. 1970. Limnological studies of Lake Norviken, a eutrophicated Swedish lake. II. Phytoplankton and its production. Schweiz. Z. Hydrol. 32: 353–396.

AUER, M. T., M. S. KIESER, AND R. P. CANALE. 1986. Identification of critical nutrient levels through field verification of models for phosphorus and phytoplankton growth. Can. J. Fish. Aquat. Sci. 43: 379–388.

BARD, J. 1970. Comparison of gradient methods for the solution of non-linear parameter estimation problems. Soc. Ind. Appl. Math. J. Numer. Anal. 7: 157–180.

BEANLANDS, G. E., AND P. N. DUINKER. 1983. An ecological framework for environmental impact assessment in Canada. Federal Environmental Assessment Review Office, Hull, Que. 132 p.

BIERMAN, V. J. 1981. Phytoplankton — nutrient kinetics mechanisms in mathematical simulation models, with special attention to reservoirs and impoundments, p. 11–32. *In* M. Lorenzen [ed.] Phytoplankton – environmental interactions in reservoirs. Vol. I. U.S. Army Waterways Experimental Station, Vicksburg, MS. 379 p.

COLLINS, C. D. 1980. Formulation and validation of a mathematicl model of phytoplankton growth. Ecology 61: 639–649.

CONTOIS, D. E. 1959. Kinetics of bacterial growth: relationship between population density and specific growth rate of continuous cultures. J. Gen. Microbiol. 21: 40–50.

DITORO, D. M. 1980. Applicability of cellular equilibrium and Monod theory to phytoplankton growth kinetics. Ecol. Model. 8: 201–218.

DROOP, M. R. 1968. Vitamin B12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in Monochrysis lutheri. J. Mar. Biol. Assoc. U.K. 48: 469–733.

1973. Some thoughts on nutrient limitation in algae. J. Phycol. 9: 264–272.

GOLDMAN, J. C. 1977. Steady state growth of phytoplankton in continuous culture: comparison of internal and external nutrient equations. J. Phycol. 13: 251–258.

JORGENSEN, S. E. 1980. Lake management. *In* Water development, supply and management. Vol. 14. Pergamon Press, Oxford. 167 p.

LI, W. K. W. 1983. Consideration of errors in estimating kinetic parameters based on Michaelis–Menton formalism in microbial ecology. Limnol. Oceanogr. 28: 185–190.

MACCORMICK, A. J., O. L. LOUCKS, J. F. KOUNCE, J. F. MITCHEL, AND P. R. WEITER. 1972. An ecosystem model for the pelagic zone of Lake Wingra. Eastern Deciduous Forest Biome Report 72-122: 103 p. (As cited in Swartzman and Bentley 1979)

MARQUARDT, D. W. 1963. An algorithm for least-squares estimation of nonlinear parameters. J. Soc. Ind. Appl. Math. 11: 431–441.

MONOD, J. 1942. Recherches sur la croissance des cultures bactériennes. 2nd ed. Hermann, Paris. 211 p.

1949. The growth of bacterial cultures. Annu. Rev. Microbiol. 3: 371–394.

RHEE, G. Y. 1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Phycol. 9: 495–506.

1974. Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. J. Phycol. 10: 470–475.

SIMONS, T. J. AND D. C. L. LAM. 1980. Some limitations of water quality models for large lakes: a case study of Lake Ontario. Water Resour. Res. 16: 105–116.

SWARTZMAN, G. L., AND R. BENTLEY. 1979. A review and comparison of plankton simulation models. I.S.E.M. J. 1: 30–81.

TARAPCHAK, S. J., AND L. R. HERCHE. 1986. Phosphate uptake by microorganisms in lake water: deviations from simple Michaelis–Menton kinetics. Can. J. Fish. Aquat. Sci. 43: 319–328.

THÉRIEN, N., K. A. MORRISON, M. DE BROISSIA, AND B. MARCOS. 1982. A simulation model of plankton dynamics in reservoirs of the La Grande River complex. Naturaliste can. 109: 869–881.

WETZEL, R. G. 1975. Limnology. W. B. Saunders, Toronto, Ont. 743 p.

WILLIAMS, P. J. 1973. The validity of the application of simple kinetic analysis to heterogeneous microbial populations. Limnol. Oceanogr. 18: 159–165.

Tumors in Cetaceans: Analysis and New Findings

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We describe 14 tumors, including 5 leiomyomas, 6 papillomas, 2 adrenal adenomas, and a bronchogenic carcinoma collected from over 1800 free-ranging and captive cetaceans examined since 1973. These bring to 41 the number of confirmable tumors reported from cetaceans. Of the 52 cases previously reported, 23 were described in sufficient detail to confirm the diagnoses, 15 were considered probable, and 4 were reclassified. Organ systems most commonly affected were the gastrointestinal tract (31%), skin (24%), and female reproductive tract (21%). Sampling bias may account for this apparent trend. Etiologic agents have rarely been identified, and attempts to do so are hampered by inadequate sample sizes.

Les auteurs décrivent 14 tumeurs, dont 5 léiomyomes, 6 papillomes, 2 adénomes des surrénales et 1 carcinome bronchogène, prélevées à partir de plus de 1 800 cétacés libres ou captifs examinés depuis 1973. Cela porte à 41 le nombre de tumeurs confirmables signalées pour des cétacés. Des 52 cas déjà signalés, 23 ont été décrits de façon suffisamment détaillée pour en confirmer les diagnoses, 15 ont été considérés probables et 4 ont été reclassifiés. Les organes les plus couramment affectés étaient le tractus gastrointestinal (31 %), la peau (24 %) et le tractus génital femelle (21 %). Cette tendance apparente peut d'expliquer par des biais de prélèvement. Les agents étiologiques ont rarement été identifiés et les tentatives dans ce domaine se voient limitées par la taille insuffisante des échantillons.

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Fifty-two tumors in cetaceans (whales, dolphins, and porpoises) have been described in 24 reports (Table 1). There has been a tendency in recent reviews (Landy 1980; Howard et al. 1983) to treat all of these "tumors" as confirmed neoplasms. In fact, many are not well documented and the reader is left to judge the validity of the diagnosis without the evidence required to meet current criteria.

In this report, we examine prevalence, morphologic characteristics, site specificity, and accuracy of diagnosis of all neoplasms reported in cetaceans. In addition, we add 14 tumors which we have gathered from over 1800 cetaceans examined since 1973.

Lipoma

A few lipomas have been reported from the musculature (Cockrill 1960) and liver (Migaki and Albert 1982) of whales and are consistent with findings in these locations in dogs and cats, respectively (Stannard and Pulley 1978). Pilleri's (1966) report of a cerebral lipoma in a humpback whale (*Megaptera novaeangliae*) is a fortuitous finding stemming from his particular interest in brain anatomy. Lipomas may occur on the serosal surface of visceral organs, or in omentum. The small pedunculated balls of adipose tissue which Cockrill (1960) mentioned from these locations may be lipomas, or could be nodules of depot fat of the type often seen in domestic animals.

Fibroma

Fibromas are the tumors most commonly reported from cetaceans, yet many of the diagnoses are questionable. Stolk's (1953) variable findings of ulceration, prominent blood vessels, necrosis, edema, and inflammatory cells in nodular lesions in the skin of humpback, fin (*Balaenoptera physalus*), and sperm (*Physeter catodon*) whales seem more appropriately to describe granulation tissue than fibromas as he suggested. Similarly, his reported fibromas on the tongue of a fin and humpback whale (Stolk 1952) appear on the basis of descriptions, illustrations, and more recent findings (Greenwood et al. 1974; this study, Fig. 1) to be fibropapillomatous growths. The appearance of mononuclear cells in considerable numbers also calls into question Mawdesley-Thomas' (1974) diagnosis of fibromas in the pleura of fin and blue whales (*Balaenoptera musculus*). Without histological support, Clarke's (1956) "hard fibroma" on the jaw of a sperm whale is also uncon-

TABLE 1. Tumors reported from cetaceans.

System	Location	Tumor	Species	Age	Sex	Source	Reference	Confirmation[a]
Integument	Skin	Verruca	Sperm whale	Imm.	M	Antarctic	Stolk 1953	A
	Lip	Melanoma	Sei whale	Mat.	F	South Africa	Uys and Best 1966	A
	Penis	Papilloma	Killer whale	imm.	M	Captive	Greenwood et al. 1974	A
	Skin	Squamous cell carcinoma	Pacific white-sided dolphin	Ukn.	Ukn.	Captive	Howard et al. 1983	A
	Penis	Papilloma	Atlantic white-sided dolphin		M	Maine	This report	A
	Skin	Papillomas (3 cases)	Narwhal, harbor porpoise, killer whale	—	M,F	See Table 3	This report	A
	Skin/ dorsal fin	Papilloma	Killer whale	Ukn.	Ukn.	Captive	Greenwood et al. 1974	B
	Penis	Papilloma	Harbor porpoise	Mat.	M	Ukn.	Landy 1980	B
	Lower jaw	Fibroma	Sperm whale	Ukn.	Ukn.	Azores	Clarke 1956	C
	Lower jaw	Fibroma	Sperm whale	Mat.	M	Antarctic	Stolk 1952	B
	Skin	Fibroma	Fin whale	Ukn.	Ukn.	Antarctic	Stolk 1952	B
	Skin/ lower jaw	Fibroma	Humpback whale	Mat.	F	Antarctic	Stolk 1953	D-?granulation tissue
	Skin	Fibroma	Fin whale	Mat.	F	Antarctic	Stolk 1953	D-?granulation tissue
	Skin	Fibroma	Sperm whale	Imm.	M	Antarctic	Stolk 1953	D-?granulation tissue
Gastrointestinal	Liver	Lipoma	Bowhead whale	Imm.	M	Barrow, AK	Migaki and Albert 1982	A
	Liver	Hemangioma	Sperm whale	Imm.	M	Antarctic	Stolk 1953	D-?hemangio-sarcoma
	Liver	Reticulo-endotheliosis	Bottlenose dolphin	Ukn.	F	Ukn.	Landy 1980; Ridgway, pers. comm.	?
	Liver	Adenoma	Bottlenose dolphin	Mat.?	M	Netherlands coast	Stolk 1952	D-?parasitic lesion, nodular hyperplasia
	Liver serosa	Lipoma	Blue whale	Mat.	F	Antarctic	Cockrill 1960	D
	Pancreas	Carcinoma	Bottlenose dolphin	Mat.	M	Ukn.	Landy 1980; Taylor and Greenwood, pers. comm.	B
	Tongue	Squamous cell papilloma	Blue whale	Ukn.	Ukn.	Antarctic	Rewell and Willis 1949	A
	Tongue/ pharynx	Papilloma (2 cases)	Atlantic white-sided dolphin		Both M	Maine	This report	A
	Tongue	Fibroma	Humpback whale	Mat.	F	Antarctic	Stolk 1952	D-?papilloma
	Tongue	Fibroma	Fin whale	Mat.	F	Antarctic	Stolk 1952	D-?papilloma
	Gingiva	Fibroma	Pacific white-sided dolphin	Mat.	F	Ukn.	Howard et al. 1983	A
	Intestine	Leiomyoma (5 cases)	Atlantic white-sided dolphin	Mat.	All F	Maine	This report	A
	Intestine serosa	Lipoma	Blue whale	Mat.	Ukn.	Antarctic	Cockrill 1960	B
	Stomach Serosa	Lipoma	Blue whale	Mat.	F	Antarctic	Cockrill 1960	B
Reproductive (female)	Uterus	Fibromyoma	Ukn. (fin or blue)	Ukn.		Antarctic	Rewell and Willis 1949	A
	Uterus	Fibromyoma	Sperm whale	Mat.		South Africa	Uys and Best 1966	A
	Uterus	Fibromyoma	Blue whale	Mat.		Antarctic	Stolk 1950	B
	Uterus	Leiomyoma calcified	Pilot whale	Mat.		Newfoundland	Cowan 1966	B
	Uterus	Fibromyoma	Ukn.	Ukn.		Ukn.	Slijper 1949, cited in Mawdesley-Thomas 1974	B
	Vagina	Fibromas	Beaked whale	Mat.		California	Flom et al. 1980	A
	Ovary	Mucinous cystadenoma	Blue whale	Mat.		Antarctic	Rewell and Willis 1949	A
	Ovary	Granulosa cell tumor	Blue whale	Mat., preg.		Antarctic, S. Georgia	Rewell and Willis 1949	A

TABLE 1. *(Continued)*

System	Location	Tumor	Species	Age	Sex	Source	Reference	Confirmation[a]
	Ovary	Granulosa cell tumor	Fin whale	Ukn.		Antarctic	Rewell and Willis 1949	A
	Ovary	Granulosa cell tumor	Fin whale	Mat., preg.		Antarctic, S. Georgia	Rewell and Willis 1949	A
	Ovary	Carcinoma	Fin whale	Mat.		Antarctic	Stolk 1950	D-?granulosa cell tumor
	Ovary	Granulosa cell tumor	Pilot whale	Mat.			Benirschke and Marsh 1984	A
Nervous/ sensory	Mediastinum	Ganglio-neuroma	Blue whale	Ukn.	Ukn.	Ukn.	Rewell and Willis 1950	A
	Cerebrum/ cerebellum	Neurofibroma	Fin whale	Mat.	Ukn.	Ukn.	Pilleri 1968	A
	Junction of pons and medulla	Lipoma	Humpback whale	Mat.	M	Ukn.	Pilleri 1966	A
Endocrine	Adrenal	Adenoma (2 cases)	Atlantic white-sided dolphin	Mat.	Both F	New England coast	This report	A
	Adrenal	Ukn.	Bottlenose dolphin	Ukn.	Ukn.	Ukn.	Mawdesley-Thomas 1974	B
Urinary	Bladder (w/metast.- lungs, intestine)	Transitional cell carci-noma	Beluga whale	Imm.	M	St. Lawrence, Quebec	Martineau et al. 1985	A
	Kidney	Adenoma	Bottlenose dolphin	Mat.	M	Gulf of Mexico	Migaki et al. 1978	A
Respiratory	Lung	Squamous cell carcinoma	Amazon porpoise				This report	A
	Lungs	Reticuloendo-theliosis	Bottlenose dolphin	Ukn.	F	Ukn.	Landy 1980; Ridgway, pers. comm.	?
Reproductive (male)	Testicle	Leydig cell	Common dolphin	Ukn.	M	California	Cowan et al. 1986	A
	Testicle	Ukn.	Bottlenose dolphin	Ukn.	M	Ukn.	Mawdesley-Thomas 1974	B
Muscle/ connective tissue skeleton	Dorsal muscle	Lipoma	Fin whale	Ukn.	Ukn.	Antarctic	Cockrill 1960	B
	Pleura	Fibroma	Blue whale	Ukn.	Ukn.	Ukn.	Mawdesley-Thomas 1974	D-?depot fat
	Pleura	Fibroma	Fin whale	Ukn.	Ukn.	Ukn.	Mawdesley-Thomas 1974	D-?depot fat
	Glenoid fossa	Osteoma	Fin whale	Ukn.	Ukn.	Ukn.	Mawdesley-Thomas 1974	D-?exostosis
Blood	Spleen, lymph nodes	Lympho-sarcoma	Pacific white-sided dolphin	Mat.	M	Ukn.	Howard et al. 1983	A
	Spleen, liver, lymph nodes, kidney	Eosinophilic leukemia	Pacific white-sided dolphin	Mat.	M	Ukn.	Howard et al. 1983	A
	Spleen, var. lymph nodes, marrow, liver	"Hodgkin's disease"	Fin whale	Mat.	M	Antarctic	Stolk 1962	B

TABLE 1. *(Concluded)*

System	Location	Tumor	Species	Age	Sex	Source	Reference	Confirmation[a]
	Spleen	Lympho-sarcoma	Bottlenose dolphin	Mat.	F	Ukn.	Landy 1980; Taylor and Greenwood, pers. comm.	B

[a]A, confirmed; B, probable, unconfirmed; C, doubtful, unconfirmable; D, disputed.

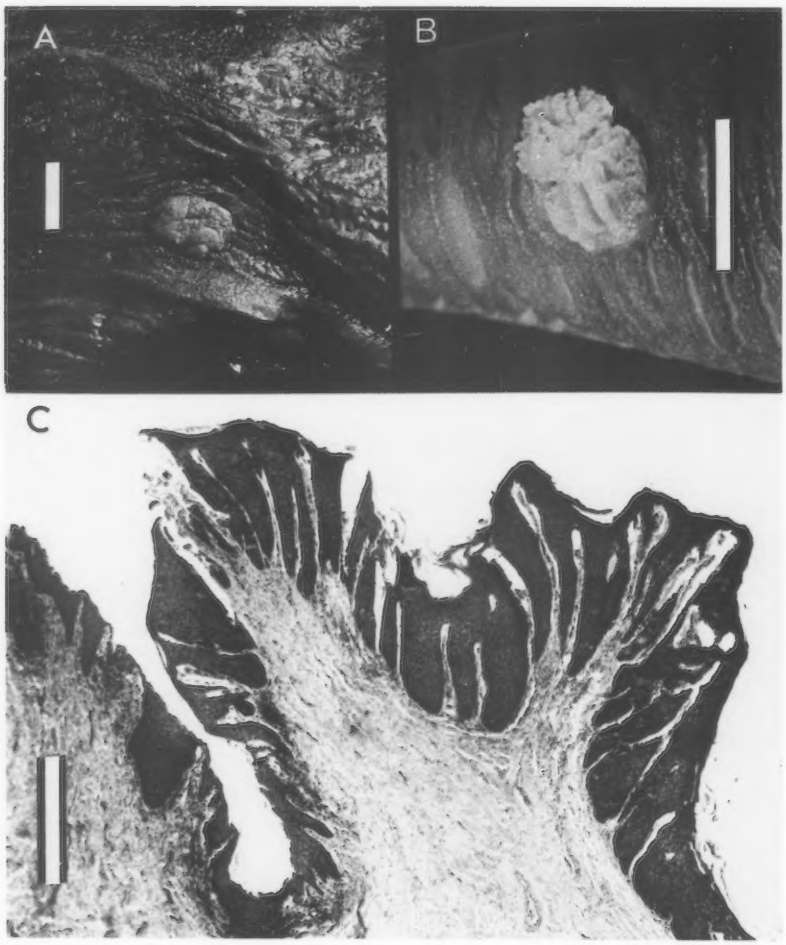

FIG. 1. Fibropapillomas on the (A) tongue and (B) penis of Atlantic white-sided dolphins. Scale bar = 1 cm (A and B). (C) Histologically, the irregular protrusion on the penis consisted of relatively normal epidermis overlying a core of fibrous tissue. Scale bar = 0.5 mm.

firmed. Thus the fibromas which can be substantiated are reduced to 2 of 13 published cases: one each on the gingiva (Howard et al. 1983) and vagina (Flom et al. 1980) of small odontocetes.

These tumors are harmless and have no known malignant potential. They are important merely to illustrate how questionable diagnoses can be transcribed through successive publications, eventually to become established as fact (Landy 1980; Flom et al. 1980; Howard et al. 1983).

Neurofibroma

Neurofibromas, also known as Schwannomas, are consid-

TABLE 2. Leiomyomas recovered from a survey of 41 Atlantic white-sided dolphins (*Lagenorhynchus acutus*) mass-stranded along the coast of Maine (Geraci et al. 1978).

Age	Sex	Tumor location[a]	Size (cm)	Number	Remarks
15.5	F	0.6/44	3.0, 1.0	2	Few large vascular spaces in tumor
5.5	F	Not recorded	0.7, 1.0	2	Decreased nuclear density on serosal side
12	F	26/34	0.5	1	Decreased nuclear density on serosal side
8	F	21/45	1.5	1	Few perivascular lymphocytes at periphery of tumor
12	F	22/35	1.0	1	Protruding into outer muscle layer, still benign

[a]Distance in metres from pylorus/total length of intestine.

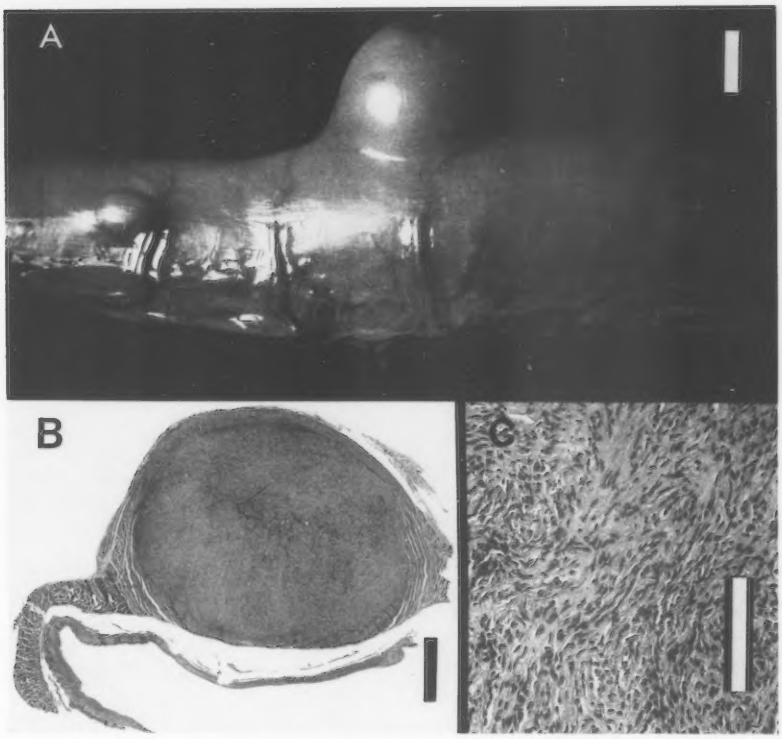

FIG. 2. (A) Firm nodules were found in the wall of the small intestine in five female Atlantic white-sided dolphins. Scale bar = 1 cm. (B) The growths typically involved the inner muscular layer, but did not penetrate the mucosal surface. Scale bar = 2 mm. (C) Histologically, smooth muscle cells were interwoven in undulating bundles. Scale bar = 50 μm.

ered by some to be associated with infection by a virus (Doughty 1972). They occur commonly in the brachial plexus, intercostal nerves, and cardiac nerves of cattle (Monlux and Monlux 1972) and cranial and spinal nerve roots of dogs. The occurrence of two neurofibromas in the central nervous system near the paraflocculus ventralis of the cerebellum in a fin whale is therefore unusual (Pilleri 1968). Both tumors were compact and did not impinge upon adjacent neural tissue. Histologically, they were rich in collagen and fibroblasts, with no mitoses or degenerative changes, which is consistent with a diagnosis of benign neurofibroma. The tumor was thought to be of embryonic origin.

Osteoma

Osteomas are rare and occur most often in bones of the head. Many of the nonmalignant bony masses that develop in

TABLE 3. Papillomas noted in free-ranging and captive cetaceans.

Species	Source	Age	Sex	Tumor location	Remarks
Lagenorhynchus acutus	Maine, 1974, stranding	8.5	M	Pharynx	Fibropapilloma
Lagenorhynchus acutus	Maine, 1974, stranding	Imm.	M	Tongue	Fibropapilloma
Lagenorhynchus acutus	Maine, 1974, stranding	12	M	Penis	Fibropapilloma
Monodon monoceros	Arctic, native hunt	(299 cm)	M	Mid-dorsal ridge	Squamous papilloma
Phocoena phocoena	Bay of Fundy, incidental take	5	M	Insertion of pectoral flippers	Squamous papilloma
Orcinus orca	Captive	6	F	Base of fluke	Squamous papilloma

and around the animal skeleton are produced by reactive rather than neoplastic osteoblasts. In some cases the lesions are traumatic in origin. The differential diagnosis of these proliferative bony lesions requires precise clinical details and critical microscopic examination.

Mawdesley-Thomas (1974) presented a photomicrograph, with no other description, of an osteoma of the glenoid fossa in a fin whale. Based on the unusual location of the lesion, and insufficient documentation, we question the validity of the diagnosis.

Leiomyoma/Fibromyoma

Five leiomyomas are the only neoplasms described in the cetacean uterus; none has been reported from any organ in a male. Gross and microscopic descriptions of the uterine tumors are consistent with established diagnostic criteria. This includes Cowan's (1966) brief mention of a calcified leiomyoma in the uterus of a pilot whale (*Globicephala melaena*). Calcification is regarded as an end-stage lesion in this tumor in older women.

Leiomyomas also occur in the wall of the small intestine where they can protrude toward the inner surface, causing obstruction. In our own collection, we observed seven such tumors on the small intestine of 5 of 25 adult female Atlantic white-sided dolphins (*Lagenorhynchus acutus*) which stranded as a group along the New England coast in 1974 (Geraci et al. 1978) (Table 2; Fig. 2). The tumors were roughly spherical masses, 0.5–3.0 cm in diameter, that protruded from the serosal surface at various points in the proximal two thirds of the intestine. Microscopically they were unencapsulated, discrete nodules located in the inner circumferential layer of the muscle wall. The cells, typical uniform smooth-muscle cells with sausage-shaped nuclei, were arranged in gently interwoven undulating bundles. Mitoses were absent. Two of the tumors were growing through the outer muscle layer of the intestine towards the serosa but maintained a benign appearance.

Papilloma

Papillomas have been noted on the skin, the surface of the penis, and the tongue of mysticetes and odontocetes. The tumors are usually self-limiting, raised, fissured horny masses which are often attached by a stalk. Two general types are recognized — squamous papilloma and fibropapilloma — although individual lesions may have features common to both. In many domestic species these lesions may be associated with host- and site-specific papovaviruses. Experimental inoculation of the virus can produce either form of the lesion. Not all papillomas are proven to be viral in origin, although the etiologic diagnosis is difficult because the virus will not grow in culture and is not easily demonstrated microscopically.

In cetaceans, five tumors representing both forms of papillomas have been reported. To this list we might add two cases, which Stolk (1952) described as fibromas of the tongue, that are more appropriately classified as fibropapillomas based on histological features. In our own collection, we have noted papillomas on the tongue (two cases) and penis (one case) of Atlantic white-sided dolphins and on the skin of a harbor porpoise (*Phocoena phocoena*) (submitted by D. Gaskin, Department of Zoology, University of Guelph, Guelph, Ont.), a narwhal (*Monodon monoceros*) (submitted by K. Hay, Department of Fisheries and Oceans) and a killer whale (*Orcinus orca*) (submitted by C. Godsell, Marineland, Niagara Falls, Ont.) (Table 3; Fig. 1 and 3). All of the lesions have the characteristic features of those described from domestic animals.

Three of the squamous papillomas were characterized by massive overgrowth of squamous epithelium and scant fibrous stroma. Grossly, these were pale lesions up to 2 cm across with multiple papillae about 1 mm thick and up to 8 mm long projecting above the epidermal surface. The base of the tumor extended as a solid mass about 2 cm below the epidermal surface and extended a few millimetres into the dermis. Germinal cells formed a narrow zone at the base of the tumor; the rest was composed of cells characteristic of stratum intermedium. Tumor cells in two of these squamous papillomas contained inclusions that suggested a viral etiology. Virus-like particles were noted on electron microscopic examination of the lesion from the harbor porpoise (Fig. 3), but not on those from killer whales (Greenwood et al. 1974; I. K. Barker and Z. Wojcinski, Department of Pathology, University of Guelph, Guelph, Ont., pers. comm.). The other three papillomatous growths in our collection were fibrous. Two were located on the tongue and pharynx and the other on the penis (Fig. 1). Each was a nodular protrusion roughly 1 cm in diameter, with a cauliflower surface. Microscopically they were composed of relatively thin pigmented epithelium cover-

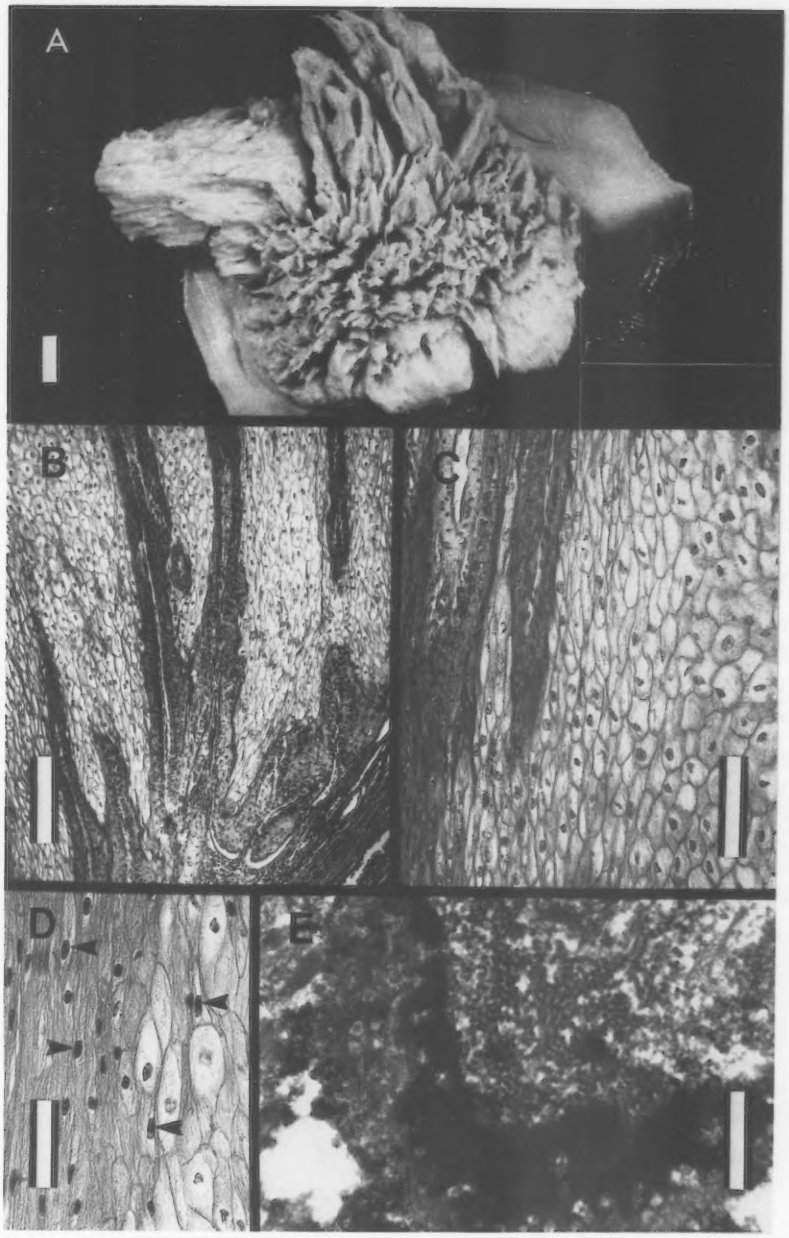

FIG. 3. (A) Squamous papilloma on the skin of a harbor porpoise. Scale bar = 1 cm. (B and C) Histologically, the tumor consisted of a massive overgrowth of squamous epithelium overlying a scant fibrous stroma. Scale Bar = 100 μm (B) and 50 μm (C). (D) Intranuclear inclusions (arrows) were evident in affected cells. Scale bar = 25 μm. (E) Virus-like particles were noted on electron microscopic examination. Scale bar = 100 nm.

ing papillary fibrous tissue cores. No suggestion of viral etiology was found in this type of tumor.

Melanoma

A small melanotic nodule was collected from the lip of a mature sei whale (*Balaenoptera borealis*) taken in South African waters (Uys and Best 1966). It was diagnosed histologically as a benign melanoma confined to the dermis. In contrast with the lesion in the sei whale, melanomas of the lip and oral cavity of dogs are invariably malignant, highly invasive, and generally fatal.

An unusual feature of the tumor in the whale was the

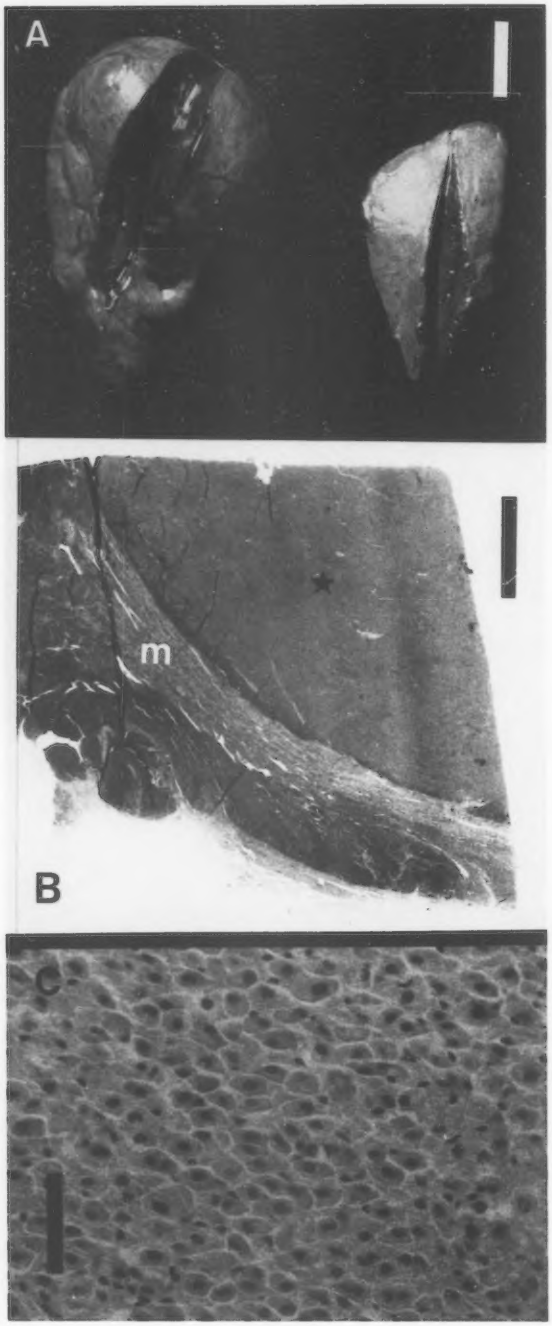

FIG. 4. (A) Unilateral adrenal enlargement due to cortical adenoma in an Atlantic white-sided dolphin. Scale bar = 2.5 cm. (B) lightly encapsulated nodule (star) compressed the adjacent medulla (m). Scale bar = 2 mm. (C) Tumor cells were polygonal to cuboidal, with abundant cytoplasm and a small irregular nucleus. Scale bar = 100 μm.

prominent development of well-formed blood vessels. This was taken as an indication that the lesion might be a hamartoma involving melanocytes and blood vessels. Such an overgrowth of vascular elements could conceivably be classified as a hamartoma, but to do so would suggest that melanocytes are normal inhabitants of the dermis.

Hemangioma

Stolk's (1953) report of a hemangioma in the liver of a sperm whale is, on the basis of his description, more suggestive of a hemangiosarcoma. The tumor was large (20 cm), invasive, had a connective tissue capsule, and an associated inflammatory cell reaction. His description did not provide details of endothelial cell morphology, but noted that there was extension of endothelium-lined spaces into adjacent liver tissue, a feature that is consistent with hemangiosarcoma. Metastases characteristic of hemangiosarcoma were not noted, but could have been overlooked by the whaling crew who collected the sample.

Ganglioneuroma

Rewell and Willis (1950) examined several slices from a mass in the anterior mediastinum of a blue whale. Their diagnosis of ganglioneuroma was based on the characteristic presence of large bundles of nerve fibers and ganglion cells embedded in a matrix of collagen fibers. This was a fortuitous finding, as this tumor is rarely encountered in domestic animals. The authors considered it to be of embryonic origin.

Renal Adenoma

Migaki et al. (1978) described an adenoma of the kidney in an aged male Atlantic bottlenose dolphin (*Tursiops truncatus*) stranded near St. Petersburg, Florida. The tumor was a 2.5-cm, firm light-brown nodule in the cortex at one pole of the kidney. The encapsulated mass was formed by well-differentiated cuboidal epithelial cells with a tubular arrangement. There was no evidence of metastasis to regional lymph nodes, and microscopically the tumor was benign.

Adrenal Adenoma

Adrenal adenomas appear as discrete yellow nodules in the adrenal gland. The cells closely resemble normal adrenal cells such as those found in hyperplastic or regenerative nodules. The diagnosis is therefore often arbitrary and based on size, number of nodules, and presence of a capsule. Adenomas usually are single nodules larger than 1−2 cm in diameter and at least partly encapsulated (Capen 1985).

We found adrenal adenomas in two Atlantic white-sided dolphins (Fig. 4). One nodule in the left adrenal of a 10.5-yr-old female measured 5.5 × 4.9 cm, was lightly encapsulated, and formed by large polygonal to cuboidal cells with abundant cytoplasm and a small regular nucleus. The tumor developed in one side of the cortex and compressed adjacent cortex and medulla. The second tumor in a mature animal was 1.5 cm in diameter, had a light capsule, and cellular morphology similar to the first. It also compressed the adjacent normal tissue.

Mawdesley-Thomas (1974) listed "adrenal cortical tumours" in *Tursiops*. His photograph shows an adrenal enlargement which could be an adrenal adenoma. Neither gross nor microscopic description was given, and the age, sex,

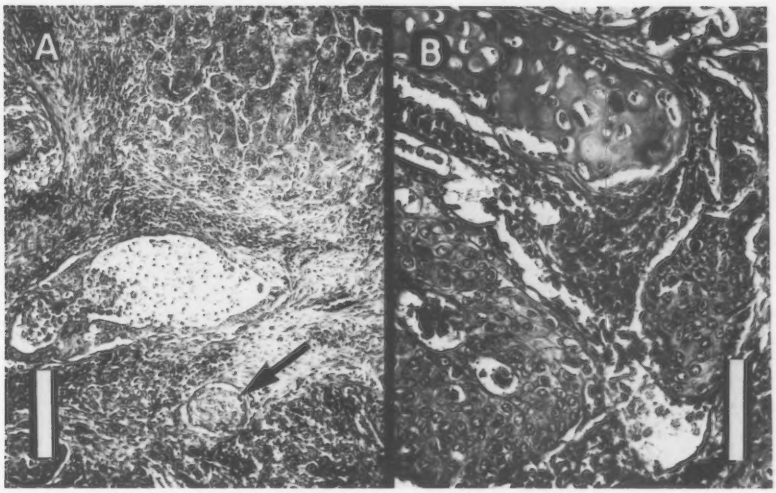

FIG. 5. Bronchogenic carcinoma in the lung of an Amazon River dolphin. (A) Neoplastic cells line a bronchiole (center), infiltrate peribronchiolar lymphatics (arrow), and replace alveolar tissue (top). Scale bar = 100 μm. (B) Around the bronchiole, tumor cells penetrated cartilagenous rings. Scale bar = 50 μm.

and source of the animal(s) are unknown. His adrenal tumors are therefore unclassifiable.

Hepatic Adenoma

Stolk (1952) found, in the liver of a bottlenose dolphin, a 24 × 20 × 13.5 mm adenoma (hepatoma) which contained many nodules about 4 mm in diameter. The granular structure was composed of tubular elements resembling bile ducts and fibrous tissue. Adjacent liver was compressed and necrotic.

Mawdesley-Thomas (1974) rejected Stolk's diagnosis. Hepatomas are usually single nodules that grossly and microscopically resemble relatively normal liver. They do not contain bile ducts. Although they may have a light capsule, fibrous tissue is sparse in the tumor itself. We believe that Stolk's gross and microscopic findings more likely represent nodular regeneration in a liver with biliary fibrosis.

Ovarian Tumors

Ovarian neoplasms reported from cetaceans are a cystadenoma and five granulosa cell tumors. No tumor of germ cell origin has been observed.

Rewell and Willis (1949) examined half of a multiloculated cystic mass about 30 cm across that was taken from the ovary of a blue whale. The outer part of the mass was fibrous ovarian stroma containing corpora albicantia with no maturing follicles or corpora lutea. Central to this, small cysts containing gelatinous fluid were separated by thin, translucent fibrous walls. Microscopically the cysts were lined by columnar mucus-secreting epithelium and stratified polygonal-celled epithelium. The center of the mass was degenerate. This tumor, which was diagnosed as mucinous cystadenoma, resembled the human rather than the canine type.

Rewell and Willis (1949) described three granulosa-cell tumors in whales killed in Antarctic waters near South Georgia. One, from a pregnant blue whale, was a round mass 3 cm in diameter with a fibrous capsule. Another, from a fin whale, was a "large follicle from ovary containing a solid body." A third, from a pregnant fin whale, resembled a fibrous cyst covered with peritoneum and contained many small cysts and abundant fibrous tissue. The microscopic appearance of the second tumor was illustrated, and as Rewell and Willis (1949) observed, closely resembled granulosa-cell tumors in other mammals. Two of three affected whales were pregnant, suggesting that any hormone production was functionally insignificant.

In a survey of 298 female reproductive tracts, Benirschke and Marsh (1984) found a granulosa-cell tumor within the right ovary of a short-finned pilot whale (*Globicephala macrorhynchus*) aged 34.5 yr (estimate). The spherical tumor was buff-colored, noncystic, about 1 cm in diameter, with occasional pseudofollicles similar to Call–Exner bodies. The authors observed that this animal was nearing the end of her reproductive years, falling into the age group in which granulosa-cell tumors most often occur in other species.

Stolk (1950) described a carcinoma of the ovary in a "very old" fin whale. The tumor consisted of a 3-cm-diameter nodule with which two smaller nodules had grown together. In a vague histologic description, Stolk referred to bladder-shaped cells with pleomorphic nuclei; some were degenerate and mineralized. Abundant, well-vascularized connective tissue containing hyaline bodies separated aggregates of these cells. Mawdesley-Thomas (1974) thought this neoplasm was a granulosa-cell tumor and we agree.

Testicular Tumors

Interstitial (Leydig) cell tumors are the most common testicular neoplasms in dogs and are typically found in older age groups. Cowan et al. (1986) diagnosed a 2.5-cm-diameter nodule from the testicle of a stranded common dolphin (*Delphinus delphis*) as a Leydig cell tumor and provided a photomicrograph to support their diagnosis. Mawdesley-Thomas' (1974) report of a testicular tumor in a bottlenose dolphin cannot be confirmed, since the only documentation provided was a gross photograph of an enlarged testicle.

Carcinoma

A single case of transitional cell carcinoma (TCC) was reported in a male beluga whale (*Delphinapterus leucas*) (Martineau et al. 1985) found dead on the shore of the St. Lawrence River, about 420 km northeast of Quebec City. In the lower urinary tract, a 5-mm-long nodule in the left ureter and an ulcer on a slightly raised mucosal patch at the apex of the bladder were shown to be foci of TCC. Multiple 1- to 8-mm nodular metastases were present throughout the lungs and a single 2 × 1 mm mass was found on the subserosa of the proximal intestine. Several microscopic metastases were present in glomeruli of a kidney. The ureteral nodule was located in the lamina propria and muscular wall, rather than the urothelium, and therefore was probably an hematogenous metastasis from the pulmonary metastases.

The diagnosis of TCC was convincing and was supported by photomicrographs. Unfortunately, an ovoid mass weighing 660 g near the right ventricle, three firm yellow nodules in a branch of the mesenteric artery, and a radiographically demonstrable lesion in the skull were not examined microscopically. Martineau et al. (1985) implied that the TCC in this beluga was caused by exposure to polynuclear aromatic hydrocarbons from an aluminum reduction plant in the area.

We found a bronchogenic carcinoma of epidermoid type in a lung specimen collected from a free-ranging Amazon River dolphin (*Inia geoffrensis*) (submitted by R. Best, Depto. de Biologia de Mamiferos Aquaticos, Brazil). The tumor was a single firm, irregular mass about 1.5 cm in diameter. Microscopically, it was composed of irregular nodules and columns of well-defined polygonal to ovoid cells with abundant pale cytoplasm (Fig. 5). Mitoses were common. The tumor was locally invasive and was destroying the lung as it spread down and through the airways. No keratinizing cells were seen but the tumor, which was clearly malignant, was typical of an epidermoid carcinoma.

Hematopoietic and Lymphoid Tissues

Howard et al. (1983) described a lymphoid neoplasm in a mature male Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). They saw marked enlargement of the spleen and mesenteric lymph nodes, and microscopically the normal appearance of these organs was replaced primarily by small to medium-sized neoplastic lymphocytes. In the lymph nodes there was an increase in fibrous tissue. Some neoplastic cells had also colonized the liver. The tumor was classified as a sclerosing malignant lymphoma.

The same authors described a myeloid neoplasm in another adult Pacific white-sided dolphin that had moderate enlargement of abdominal lymph nodes, liver, and spleen. Microscopically they found numerous eosinophils and eosinophil precursors in the spleen, liver, lymph nodes, and kidneys. Bone marrow and blood were not examined. A diagnosis of "neoplastic proliferation of the eosinophil granulocyte, and leukemia-eosinophilic" was made. The possibility that this was a myeloproliferative disease of eosinophils was also considered.

Stolk (1962) reported a diagnosis of Hodgkin's disease in an adult male fin whale. At postmortem examination, consolidation of caudal lungs and enlargement of hilar lymph nodes were found. The liver contained many white foci and the spleen was enlarged with prominent follicles. Many lymph nodes, including pelvic, mediastinal, mesenteric, and retroperitoneal, were enlarged. On section, the nodes were homogeneous, pale gray, moist, and translucent. A microscopic diagnosis of Hodgkin's disease was made, based on the presence of numerous epithelioid cells and Sternberg–Reed cells.

Hodgkin's disease is a term that refers to a condition(s) in people that is characterized by the presence of Sternberg–Reed cells in an appropriate cellular background. The condition is sometimes called Hodgkin's lymphoma but there is debate about whether it is a neoplasm or a chronic inflammatory disease. Most favor neoplastic disease (Ashley 1978; Robbins and Cotran 1979). Criteria for diagnosis are stringent, as Sternberg–Reed cells are found in conditions other than Hodgkin's disease (Strum et al. 1970). In lower animals, lymphoid neoplasms that resemble Hodgkin's disease occur (Smith and Barker 1983; Valli 1985) but they are most uncommon. We were unable to examine Stolk's original illustrations. Mawdesley-Thomas (1974) apparently did so, and while deploring Stolk's (1962) diagnostic criteria, nevertheless retained the diagnosis in his report.

Unverified

There are a few reports of tumors which we cannot classify or verify due to insufficient information. Landy (1980) listed a carcinoma of the pancreas, thyroid adenoma, reticuloendotheliosis, and lymphosarcoma in bottlenose dolphins and cited personal communications as the sources. A "yellow growth" on the flukes of a Pacific white-sided dolphin was noted by Sullivan and Houck (1979), but no specific diagnosis was made. Without the necessary documentation, we omit these cases from our compilations of tumors in cetaceans.

Discussion

Of the 52 tumors reported in cetaceans, 23 were sufficiently detailed to confirm the proposed diagnoses. We considered an additional 15 to have been inadequately described, but accept the proposed diagnoses in view of the nature of similar confirmed tumors in those locations; for example, papillomas on the skin of a killer whale (Greenwood et al. 1974) and the penis of a harbor porpoise (Landy 1980). We dispute 12 diagnoses of which 4, in fact, are tumors to which we reassigned a diagnosis by combining original descriptions and illustrations with contemporary information on etiology and classification. Adding 14 of our own cases, we now document 41 confirmable tumors.

For reasons which are as yet unclear, most animal species are predisposed to tumors at particular sites (Schneider 1978). In cetaceans, 10 neoplasms or 24% of those reported occurred in the skin, 13 (31%) were in the gastrointestinal tract, and 9 (21%) in the female reproductive system. The three systems account for over 75% of the total reported.

Rather than suggesting an apparent susceptibility of the systems to tumors, the distribution may be more appropriately explained by sampling bias. Skin tumors are obvious for their superficial occurrence and ease of recognition. Crewmen on whaling ships in the early 1950's collected a number of such specimens which were submitted to Stolk. Of the 13 tumors in the gastrointestinal tract, 5 occurred on the tongue and gingiva and might therefore have been as visible as their counterparts on skin. Moreover, the apparent prevalence of tumors in the female reproductive tract reflects the emphasis on collecting and examining this organ system for assessing age and produc-

tivity of commercially exploited species. Perhaps female cetaceans are predisposed to tumors in the reproductive tract, but the small sample size and inconsistency in sampling effort do not allow us to reach such a conclusion.

The difficulties in performing detailed examinations of all organ systems in large mysticetes or in a mass-stranded herd of odontocetes represent still another bias which has limited the diversity of reported tumors. Growths within large parenchymatous organs and intraocular and endocrine neoplasms would not readily be detected in the short time during which a commercially captured animal is processed onboard a whaling vessel. By contrast, captive dolphins and small cetaceans which strand individually or in small groups are often examined in great detail, thus accounting for the diagnosis of more subtle conditions such as adrenal adenoma and lymphosarcoma. As observational effort increases, we expect that the variety of tumors reported in cetaceans will also increase.

Few reports on cetacean tumors propose a causal relationship. This is not inconsistent with the literature on spontaneous neoplasms in other free-ranging animals. Such relationships can only be demonstrated through epidemiological studies on large population samples which are inherently difficult to obtain. The existing literature shows that tumors are influenced by numerous exogenous and endogenous factors. Among those which have been identified are hormones, viruses, congenital and hereditary defects, and physical and chemical agents. Can cetacean tumors be linked to any of these?

Exogenous administration of estrogens can produce uterine leiomyomas experimentally (Jubb et al. 1985). Yet the evidence for hormonal influence in spontaneous leiomyomas is circumstantial and debatable (Willis 1960; Brodey and Roszel 1967). Cetaceans have unusually long gestation and lactation periods through each reproductive cycle with little if any interruption. It is tempting to speculate that related hormonal changes may be associated with the five uterine leiomyomas which have been recorded and with the intestinal leiomyomas which we observed only in mature female Atlantic white-sided dolphins. However, other studies as detailed as ours, and with greater sample sizes (Perrin et al. 1977; Benirschke and Marsh 1984), have not documented a single leiomyoma in other small odontocetes. It is clear that the presence of leiomyomas cannot be explained on the basis of hormonal factors alone.

We might also look to hormonal influences and predisposing morphological factors in the development of adrenal cortical tumors in Atlantic white-sided dolphins. In our survey of 30 mature animals, we noted a high incidence of hyperplastic nodules in the adrenal cortex. These were delineated by bands of connective tissue which in the normal gland arise from the capsule and penetrate the cortex. This architecture is apparently conducive to the formation of discrete nodules of cortical cells, which might develop into adenomas under repeated or continuous stimulation by ACTH.

The role of viruses in carcinogenesis is becoming increasingly apparent in domestic animals. Papillomas and some lymphoid tumors in particular have been linked to viral infections, and neurofibromas are also suspected to be viral in origin (Yager and Scott 1985). Proof of such association in cetaceans has not been established, although suspicious intranuclear inclusion bodies and virus-like particles have been observed in squamous papillomas. The four lymphoid tumors which have been reported from cetaceans are dissimilar in many respects, and there is no basis for suggesting any common causal relationship.

There is increasing concern that cancer in certain species can be related to environmental pollutants. The relationship is rarely direct. For example, the majority of tumors linked to chemical carcinogens are found in humans with a lifestyle which exposes them to a variety of tumor-promoting and enhancing substances (Higginson 1981). Such confounding variables are major obstacles in any epidemiological study which seeks to incriminate a single substance. Martineau et al. (1985) neglected this relationship in their simplistic attempt to link environmental polycyclic aromatic hydrocarbons with transitional cell carcinoma in a beluga whale. The association in humans is obscured by intense exposure to airborne concentrations in an industrial setting and a history of cigarette smoking in affected individuals. With what certainty can we compare these victims with a single beluga, in an environment in which concentrations of polycyclic aromatic hydrocarbons are not particularly high (Neff 1979; Picard-Berube and Cossa 1983)?

The same logic, with the same pitfall, might have been used to attempt an etiology for bronchogenic carcinoma in an Amazon River dolphin. The relationship between the tumor and cigarette smoking is clear (Auerbach et al. 1970), but not exclusive.

So little is known about cetacean tumors that it hardly seems necessary to propose causes, however tempting. It should be enough to carefully document their occurrence so that any eventual association will be based on sound information. Large populations of animals need to be examined to generate the necessary data base. In the near future we might optimistically expect to expand the spectrum of known tumors and probably confirm a viral etiology for some of the papillomatous growths.

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References

Ashley, D. J. B. 1978. Evans' histological appearances of tumours. Vol. 2. 3rd ed. Churchill Livingstone, Edinburgh, 857 p.

Auerbach, O., E. C. Hammond, D. Kirman, and L. Garfinkel. 1970. Effects of cigarette smoking on dogs. II. Pulmonary neoplasms. Arch. Environ. Health 21: 754–768.

Benirschke, K., and H. Marsh. 1984. Anatomic and pathologic observations of female reproductive organs in the short-finned pilot whale, *Globicephala macrorhynchus*. Rep. Int. Whaling Comm. 6: 451–454.

Brodey, R. S. and J. F. Roszel. 1967. Neoplasms of the canine uterus, vagina and vulva: a clinicopathologic survey of 90 cases. J. Am. Vet. Med. Assoc. 151: 1294–1307.

Capen, C. C. 1985. The endocrine glands, p. 237–303. *In* K. V. F. Jubb, P. C. Kennedy, and N. Palmer [ed.] Pathology of domestic animals. Vol. 3. 3rd ed. Academic Press, Orlando, FL.

Clarke, R. 1956. Sperm whales of the Azores. Discovery Rep. 28: 237–298.

Cockrill, W. R. 1960. Pathology of the cetacea: a veterinary study on

whales. Br. Vet. J. 116: 1–30.

COWAN, D. F. 1966. Pathology of the pilot whale, *Globicephala melaena*. Arch. Pathol. 82: 178–189.

COWAN, D. F., W. A. WALKER, AND R. L. BROWNELL, JR. 1986. Pathology of small cetaceans stranded along southern California beaches, p. 323–367. *In* M. M. Bryden and R. Harrison [ed.] Research on dolphins. Part III. Clarendon Press, Oxford.

DOUGHTY, F. R. 1972. Virus particles in a bovine neurofibroma. Aust. Vet. J. 48: 365–366, 533.

FLOM, J. O., R. J. BROWN, R. E. JONES, AND J. SCHONEWALD. 1980. Vaginal fibromas in a beaked whale, *Mesoplodon densirostris*. J. Wildl. Dis. 16: 99–102.

GERACI, J. R., S. A. TESTAVERDE, D. J. ST. AUBIN, AND T. H. LOOP. 1978. A mass stranding of the Atlantic white-sided dolphin, *Lagenorhynchus acutus*: a study into pathobiology and life history. National Technical Information Service, Washington, DC. PB289-361: 141 p.

GREENWOOD, A. G., R. J. HARRISON, AND H. W. WHITTING. 1974. Functional and pathological aspects of the skin of marine mammals, p. 73–110. *In* R. J. Harrison [ed.] Functional anatomy of marine mammals. Vol. 2. Academic Press, London.

HIGGINSON, J. 1981. Cancer — achievements, challenges, and prospects for the 1980s. Vol. 1. J. H. Burchenal and H. F. Oettgen [ed.] Grune & Stratton, New York, NY. p. 7–23.

HOWARD, E. B., J. O. BRITT, JR., AND J. G. SIMPSON. 1983. Neoplasms in marine mammals, chap. 5, p. 95–162. *In* E. B. Howard [ed.] Pathobiology of marine mammal diseases. Vol. II. CRC Press, Boca Raton, FL.

JUBB, K. V. F., P. C. KENNEDY, AND N. PALMER. [ED.]. 1985. Pathology of domestic animals. Vol. 3. 3rd ed. Academic Press, Orlando, FL. 527 pp.

LANDY, R. B. 1980. A review of neoplasia in marine ammals (Pinnipedia and Cetacea), p. 579–584. *In* R. J. Montali and G. Migaki [ed.] The comparative pathology of zoo animals. Smithsonian Institution Press, Washington, DC.

MARTINEAU, D., A. LAGACE, R. MASSE, M. MORIN, AND P. BELAND. 1985. Transitional cell carcinoma of the urinary bladder in a beluga whale (*Delphinapterus leucas*). Can. Vet. J. 26: 297–302.

MAWDESLEY-THOMAS, L. E. 1974. Some aspects of neoplasia in marine animals. Adv. Mar. Biol. 12: 151–231.

MIGAKI, G., AND T. F. ALBERT. 1982. Lipoma of the liver in a bowhead whale (*Balaena mysticetus*). Vet. Pathol. 19: 329–331.

MIGAKI, G., J. C. WOODARD, AND R. T. GOLDSTON. 1978. Renal adenoma in an Atlantic bottle-nosed dolphin (*Tursiops truncatus*). Am. J. Vet. Res. 39: 1920–1921.

MONLUX, W. S., AND A. W. MONLUX. 1972. Atlas of meat inspection pathology. Agriculture Handbook No. 367, U.S. Department of Agriculture, Washington, DC.

NEFF, J. M. 1979. Polycyclic aromatic hydrocarbons in the aquatic environment. Sources, fates and biological effects. Applied Science Publishers Limited, London. 262 p.

PERRIN, W. F., D. B. HOLTS, AND R. B. MILLER. 1977. Growth and reproduction of the eastern spinner dolphin, a geographical form of *Stenella longirostris* in the eastern tropical Pacific. Fish Bull. 75: 725–750.

PICARD-BERUBE, M., AND D. COSSA. 1983. Teneurs en benzo 3,4 pyrene chez *Mytilus edulis* L. de l'estuaire et du Golfe du Saint-Laurent. Mar. Environ. Res. 10: 63–71.

PILLERI, G. 1966. Hirnlipom beim Buckelwal, *Megaptera novaeanglia* [Cerebral lipoma in a humpback whale, *Megaptera novaeangliae*]. Pathol. Vet. 3: 341–349.

1968. Cerebrale Neurofibrome beim Finnwal, *Balaenoptera physalus* [Cerebral neurofibroma in a fin whale, *Balaenoptera physalus*]. Pathol. Vet. 5: 35–40.

REWELL, R. E., AND R. A. WILLIS. 1949. Some tumours found in whales. J. Pathol. Bacteriol. 61: 454–456.

1950. Some tumours of wild animals. J. Pathol. Bacteriol. 62: 450–452.

ROBBINS, S. L., AND R. S. COTRAN. 1979. Pathologic basis of disease. 2nd ed. W. B. Saunders, Philadelphia, PA.. 1598 p.

SCHNEIDER, R. 1978. General considerations, p. 1–15. *In* J. E. Moulton [ed.] Tumors in domestic animals. 2nd ed. University of California Press, Berkeley, CA.

SMITH, D. A., AND I. K. BARKER. 1983. Four cases of Hodgkin's disease in striped skunks (*Mephitis mephitis*). Vet. Pathol. 20: 223–229.

STANNARD, A. A., AND L. T. PULLEY. 1978. Tumors of the skin and soft tissues, p. 16–74. *In* J. E. Moulton [ed.] Tumors in domestic animals. 2nd ed. University of California Press, Berkeley, CA.

STOLK, A. 1950. Tumours in whales. Amsterdam Nat. 1: 28–33.

1952. Some tumours in whales, p. 275–278. *In* Proceedings of Koninklijke Nederlandse Akademie van Wetenschappen. Vol. 55. North-Holland Publishing Company, Amsterdam, The Netherlands.

1953. Some tumours in whales II, p. 369–374. *In* Proceedings of Koninklijke Nederlandse Akademie van Wetenschappen. Vol. 56. North-Holland Publishing Company, Amsterdam, The Netherlands.

1962. Tumours in whales III, p. 250–268. *In* Proceedings of Koninklijke Nederlandse Akademie van Wetenschappen. Vol. 65. North-Holland Publishing Company, Amsterdam, The Netherlands.

STRUM, S. B., J. K. PARK, AND H. RAPPAPORT. 1970. Observation of cells resembling Sternberg–Reed cells in conditions other than Hodgkin's disease. Cancer 26: 176–190.

SULLIVAN, R. M., AND W. J. HOUCK. 1979. Sightings and strandings of cetaceans from northern California. J. Mammal. 60: 828–833.

UYS, C. J., AND P. B. BEST. 1966. Pathology of lesions observed in whales flensed at Saldanha Bay, South Africa. J. Comp. Pathol. 76: 407–415.

VALLI, V. E. O. 1985. The hematopoietic system, p. 83–236. *In* K. V. F. Jubb, P. C. Kennedy, and N. Palmer [ed.] Pathology of domestic animals. Vol. 3. 3rd ed. Academic Press, Orlando, FL.

WILLIS, R. A. 1960. Pathology of Tumours. 3rd ed. Butterworths Publishers, London. 1002 p.

YAGER, J. A., AND D. W. SCOTT. 1985. The skin and appendages, p. 407–549. *In* K. V. F. Jubb, P. C. Kennedy, and N. Palmer [ed.] Pathology of domestic animals. Vol. I. 3rd ed. Academic Press, Orlando, FL.

Mitochondrial DNA Analysis of Pacific Northwest Populations of *Oncorhynchus tshawytscha*

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Wilson, G. M., W. K. Thomas, and A. T. Beckenbach. 1987. Mitochondrial DNA analysis of Pacific Northwest populations of *Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci. 44: 1301–1305.

Mitochondrial (mt) DNA restriction endonuclease assays of seven populations of *Oncorhynchus tshawytscha* from British Columbia and Alaska disclosed six distinct maternal (clonal) lines, of which three were represented by more than one individual. The three major lines were not population specific. Of the British Columbia fish sampled, 41 belong to clonal line A (82% of the 50 fish sampled in British Columbia). Clonal line C contained 10 fish from Alaskan sites only. Clonal line B contained 20 fish from both British Columbia and Alaska sites. Intraspecific mtDNA sequence divergence is low (less than 1% in all cases) compared with other species surveys of this sort. Comparison with protein electrophoretic studies of *O. tshawytscha* reveals similar levels of population structuring. A database of mtDNA fragment sizes produced by the 14 restriction endonucleases is included.

Des essais par endonucléases de restriction portant sur l'ADN mitochondrien (mt) de sept populations de *Oncorhynchus tshawytscha* de la Colombie-Britannique et de l'Alaska ont permis de trouver six lignées maternelles distinctes (clones) dont trois étaient représentées par plus d'un individu. Les trois lignées principales n'étaient pas exclusives aux populations. La lignée clonale A comprenait 41 poissons provenant des sites de prélèvement de la Colombie-Britannique (82 % des 50 poissons prélevés en Colombie-Britannique). La lignée clonale C comprenait 10 poissons provenant exclusivement d'Alaska. La lignée clonale B comprenait 20 poissons provenant de sites de la Colombie-Britannique et de l'Alaska. La divergence intraspécifique de la séquence de l'ADNmt était faible (moins de 1 % dans tous les cas) comparativement aux valeurs notées pour d'autres espèces au cours de relevés de ce genre. Une comparaison avec les résultats d'études électrophorétiques des protéines chez *O. tshawytscha* a montré l'existence d'une structuration semblable de la population. La communication comprend aussi une base de données des tailles des fragments d'ADNmt produits par l'utilisation de 14 endonucléases de restriction.

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Individuals of the species *Oncorhynchus tshawytscha* (chinook or king salmon) are anadromous, maturing at sea but returning to their natal streams to spawn. This life history results in some isolated breeding populations. It is important for breeding management of fish stocks to have methods of identifying various breeding populations. Ocean-caught fish can then be identified as to their spawning destination. Since certain stocks have been depleted to a greater extent than others, controlling the size of the catches from each stock is important to ensure continued survival of the species and to maintain genetic heterogeneity.

We surveyed various populations of *O. tshawytscha* from Alaska to southern British Columbia, a significant portion of the North American range (McPhail and Lindsey 1970). The variation in mitochondrial (mt) DNA sequences was sampled both within and between populations and compared with results obtained in other studies. The latter were mostly obtained by electrophoretic separation of isozymes and analysis of allelic frequences at polymorphic loci.

A second objective of this study was to provide a database of mitochondrial fragment sizes in this species for a large number of commonly used restriction endonucleases. This will provide a means of comparison with mtDNA assays of other populations of this species.

The analysis of variation in mtDNA by digestion with type II restriction endonucleases has proven to be a successful method to address questions of population structure (Lansman et al. 1981). The small size of the mitochondrial genome and ease of its isolation make this technique ideal for sampling large numbers of individuals. The apparent high rate of sequence evolution (Brown et al. 1979), maternal inheritance (Brown et al. 1979; Giles et al. 1980), and individual homogeneity (Avise et al. 1979) allow for the detection of variation within species and the organization of individuals into matriarchal phylogenies.

Materials and Methods

The fish were obtained from Robertson Creek Hatchery (N = 12), Puntledge Hatchery (N = 11), Capilano Hatchery (N = 12), Indian Point River (N = 9), Quesnel River (N = 8), Copper River (N = 12), and Bristol Bay (N = 12).

In all cases the tissue used for mtDNA extraction was the liver. Livers were removed from the fish at the sample sites and shipped on ice but not frozen. Extraction was by the method of Lansman et al. (1981). All samples were purified on cesium chloride prior to restriction.

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TABLE 1. Fragment sizes produced by restriction enzyme digestion of *O. tshawytscha* mtDNA.

			Bgl I						
Eco RI	*Bam* HI	*Pst* I	B,C,F	E	A	D	*Bgl* II	*Kpn* I	*Eco* RV
9 100	11 800	14 000	7 000	7 000	7 000	7 000	9 200	16 500	15 700
4 100	4 100	1 700				5 300	3 500		800
4 100		400	4 400	4 400	4 400	4 400	2 500		
				4 400			1 300		
			4 100						
					3 200				
			1 200		1 200				
				900	900				
			400	400	400	400			
17 300	15 900	16 100	17 100	17 100	17 100	17 100	16 500	16 500	16 500

	Hind III				*Xba* I			*Ava* II	
Sma I	A,B,C,D,E	F	*Hinc* II	*Hae* II	A,C,E,F	B,D	*Pvu* II	A,D,E,F	B,C
16 500	6 600	6 600	5 800	7 200	8 00	8 000	6 800	2 500	2 500
	3 400		2 600	4 600		5 100	4 800	2 200	2 200
	2 100	2 100	2 400	1 000	3 600		2 500	1 900	
		2 000	2 400	1 000	2 400	2 400	2 400	1 500	1 500
	1 800	1 800	1 100	1 000	1 500				1 500
		1 400	800	700	700	700		1 300	1 300
	1 200	1 200	800	700				1 100	1 100
	1 100	1 100	500					1 100	1 100
	300	300						900	900
								900	900
								600	600
								600	600
								500	500
									400
								400	400
								400	400
16 500	16 500	16 500	16 400	16 200	16 200	16 200	16 500	15 900	15 900

Restriction endonuleases used which cleave six-base recognition sequences were *Eco* RI, *Bam* HI, *Hind* III, *Bgl* I, *Bgl* II, *Kpn* I, *Pst* I, *Sma* I, *Xba* I, *Eco* RV, and *Pvu* II. Two enzymes used recognize six-base multiple sequences (*Hinc* II and *Hae* II), and one recognizes a five-base multiple sequence (*Ava* II).

Separation of mtDNA fragments was by agarose gel electrophoresis on horizontal slab gels. The gels contained 0.7–1.0% agarose and 0.5 μg ethidium bromide/mL and were run at 75–80 V for 2.5–3.0 h. mtDNA fragments were observed under ultraviolet (UV) light. Sample fragment sizes were estimated by comparison with migration distances of phage lambda fragments produced by digestion with *Hind* III or double digestion with *Hind* III and *Eco* RI.

The levels of nucleotide substitution were determined by the maximum likelihood method of Nei and Tajima (1983). The mtDNA clones were organized into a parsimony network using the Phylip programs of Joe Felsenstein.

Results

Table 1 shows all of the mtDNA fragments generated with the 14 enzymes used. Enzymes for which restriction fragment length differences (RFLD's) occur have more than one column. Each polymorphism identified by the addition or loss of a cut site is assumed to be due to a single nucleotide substitution event. An example of two alternative fragment patterns generated by the restriction enzyume *Xba* I is shown in Fig. 1. In this case, individuals 2 and 5 display a 5.15-kb fragment, which in the other individuals contains an internal *Xba* I site resulting in 3.6- and 1.55-kb fragments.

The enzymes used recognized approximately 68 cut sites (the exact number varies slightly with different clonal lines) comprising a sample of more than 400 nucleotides per mitochondrial genome. Of these cut sites only three were phylogenetically informative. These variable sites define the clonal lines, each line being composed of all of the fish which produce identical fragment patterns with each of the 14 enzymes. A total of six clonal lines were found. Table 2 lists the polymorphic cut sites and the status of each clonal line with respect to each site.

Clonal line A consists of 43 British Columbia fish and represents 79% of the fish sampled from five different sites. Of the 20 fish in clonal line B, 6 (30%) are from British Columbia sites and 14 (70%) are from Alaska. Clonal line C contains 10 fish, all of which spawn in Alaska. Clonal lines D, E, and F are minor lines, each represented by a single fish from Capilano Hatchery, Robertson Creek, and Bristol Bay, respectively.

The data is further analysed in two ways. The maximum likelihood estimates of Nei and Tajima (1983) provide a quantitative measure of the percent nucleotide substitution in pairwise comparisons (Table 3). It is of interest to note that the minimum pairwise divergence of 0.13 ± 0.13% occurs between the major British Columbia and Alaska lines A and C, respectively, as well as the lines C and F. In the case of the A

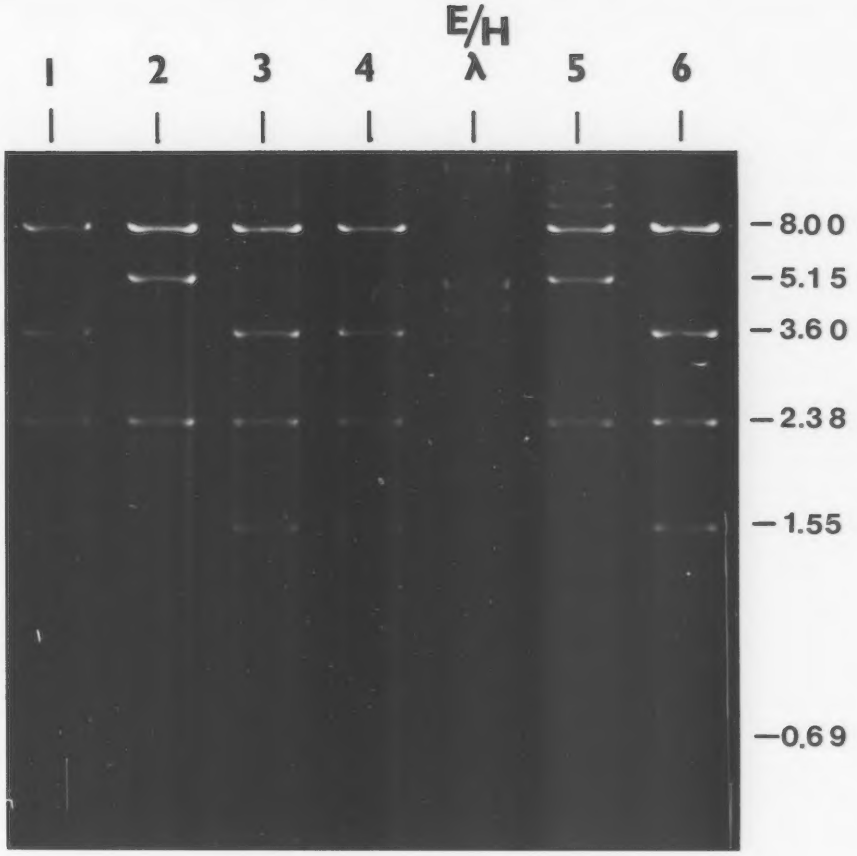

FIG. 1. *Xba* I digests of six individuals from Quesnel River. Lanes 1, 3, 4, and 6 represent clones A, C, E, and F. Lanes 2 and 5 represent clones B and D. E/H is *Eco* RI/*Hind* III digested phage lambda.

TABLE 2. Polymorphic cut sites (+ indicates the presence of the cut site).

	Clonal line					
Enzyme	A	B	C	D	E	F
Bgl I	+	+	+			+
Bgl I	+				+	
Xba I	+		+		+	+
Hind III						+
Ava II		+		+		

and C lines this divergence represents a single *Bgl* I cut site difference between the two lines. Table 3 also lists the number of cut site differences or mutational events between pairs of clones. The pairwise estimates of sequence divergence reflect the overall divergence of two genomes. The individual mutational events can also be used to organize the clones into matriarchial phylogenies following parsimony criteria.

Figure 2 is a geographic representation of a parsimony network which represents clonal lines organized to minimize the total number of cut sites separating them. This network shows the most reasonable phylogeny by the parsimony criteria. Clonal line E, a single Robertson Creek fish, does not fit neatly into this network because of apparent convergent mutations (homoplasy). If we assume that the loss of the *Xba* I site resulted from an independent nucleotide substitution from the other individuals which have lost this site, the network is resolved.

Discussion

The small number of major clonal lines (three) and low percent nucleotide substitution uncovered by this study indicate that both within- and between-population variation is low for a major portion of this species' North American range. All pairwise comparisons of nucleotide sequence divergence gave values less than 1%. The variation uncovered is not population specific but does show evidence of a geographic trend. The Wagner network superimposed on a map of the sample sites (Fig. 2) displays the geographic distribution of the clonal lines. The most closely related individuals are not always those with the smallest geographic distance separating them. Clonal lines C and A appear to be restricted to the north and south, respectively. Even clonal line B, which is present in all areas, makes up a much higher percentage of the Alaska fish (54%, 13 of 24) than the British Columbia fish (13%, 7 of 52).

TABLE 3. Pairwise sequence divergence estimates and standard deviations for mtDNA clones (above the diagonal). Minimum number of mutational events are given below the diagonal.

	A	B	C	D	E	F
A	—	0.67±0.30	0.13±0.13	0.55±0.27	0.40±0.23	0.53±0.26
B	3	—	0.54±0.27	0.40±0.23	0.81±0.33	0.67±0.30
C	1	2	—	0.41±0.23	0.54±0.27	0.13±0.13
D	4	1	3	—	0.41±0.23	0.55±0.27
E	1	4	2	3	—	0.40±0.23
F	2	3	1	4	3	—

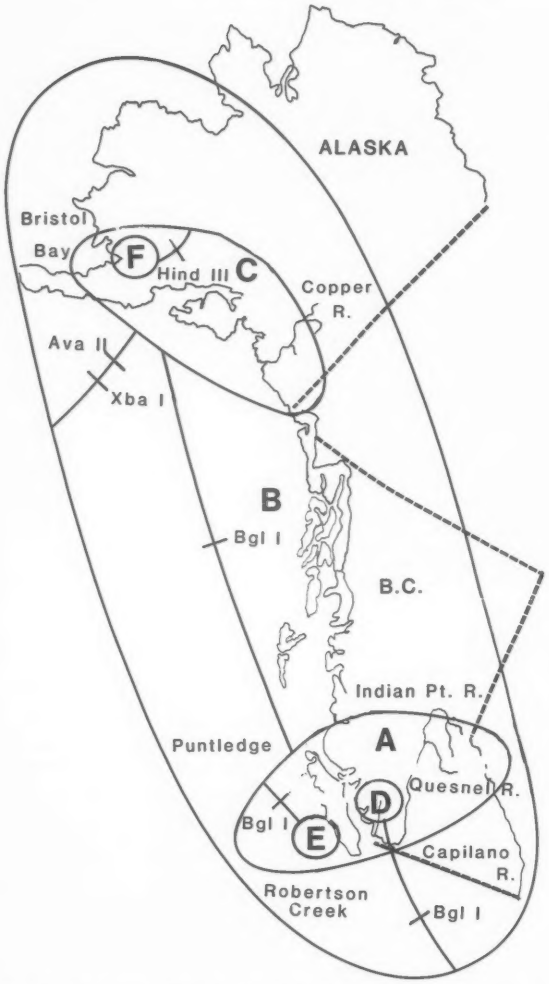

FIG. 2. Parsimony network linking the six mitochondrial genotypes of *O. tshawytscha* detected in this study. Geographic distribution of mtDNA clones A, B, and C is shown. Mitochondrial genotypes D, E, and F were detected in single fish (see text).

None of the populations from contiguous United States were analysed in this study, but Berg and Ferris (1984) studied two individuals from California. Of 10 enzymes common to both studies, cut site differences were evident in 2: *Eco* RI (two cut site differences) and *Ava* II (a minimum of six changes). These California samples, caught off the coast near San Francisco, were found to be most similar to our clonal line A with a minimum divergence estimate of 2.2%. This minimum divergence, estimated between California and northern populations of the species, is extraordinarily high when compared with the rest of the populations sampled and is almost as great as that observed between chinook and coho (*O. kisutch*) salmon (Thomas et al. 1986). If the results of Berg and Ferris, based on only two individuals, are representative of the California populations, then substantial variability separates the California and the British Columbia populations. Most of the divergence, however, is uncovered with only one enzyme, *Ava* II. The *Ava* II sequence (5′GGA/TCC) may be hypervariable, resulting in very rapid sequence divergence, and possibly inflating the overall sequence divergence estimate. Such hypervariability has been reported elsewhere (Hale and Beckenbach 1985). Another possible explanation for this high level of intraspecific divergence is the hypothesized existence of two glacial refugia for *O. tshawytscha*, the Pacific and Bering refuges. Populations from British Columbia and Alaska are thought to have been derived from fish of the Bering refuge, while populations from California are thought to have been derived from the Pacific refuge (McPhail and Lindsey 1970).

Kristiansson and McIntyre (1976) sampled 10 hatchery populations of *O. tshawytscha* from the Columbia River and Oregon coast. Of 14 proteins assayed by electrophoretic separation of isozymes, highly significant differences between Columbia River and coastal populations were uncovered at two polymorphic loci. Their results indicate a low level of variation and geographic substructuring. Estimates of population substructuring derived from allozyme studies (Utter et al. 1980) are in agreement with our results.

We also surveyed mtDNA variation in the rainbow trout (*Salmo gairdneri*) (Wilson et al. 1985). In this study, 38 rainbow trout from an area restricted to the coast of British Columbia fell into seven distinct clonal lines. These lines ranged in pairwise sequence divergence from 0.13 ± 0.13 to 0.922 ± 0.346%. Six of the seven clonal lines were each restricted to one of the nine sites sampled. The other three sites shared the seventh clonal line. This higher level of substructuring in the rainbow trout as compared with that of the chinook presented above is probably a result of the restricted potential for migration of the nonanadromous morph of the rainbow trout.

Restriction endonuclease assays of 87 individuals of the species *Geomys pinetis* (pocket gopher) revealed two major maternal lines within three states: Georgia, Florida, and Alabama (Avise et al. 1979). These two lines were at least 3% divergent in mtDNA sequence. The maximum divergence found between two lines in the chinook salmon was 0.8%, although a divergence of 2.2% between line A and the California samples of Berg and Ferris has been noted.

Fisheries Applications of mtDNA Analysis

This survey of *O. tshawytscha* mtDNA variation required the use of an expensive method of extraction in order to maximize yields and optimize the purity of mtDNA from each fish. A large number of the enzymes used to assay the mtDNA produced no restriction fragment length differences and would therefore be of no interest in fish management except in subsequent surveys of different populations. For the region sampled, use of just two enzymes, *Bgl* I and *Xba* I, will distinguish five clonal lines in the fish, including the three major lines. A third enzyme (either *Ava* II or the less expensive *Eco* RI) will distinguish the California samples of Berg and Ferris from any of our samples. For fish management applications, once diagnostic enzymes have been found as above, cheaper procedures can be used to extract and assay mtDNA as described in Lansman et al. (1981).

Although the amount of sequence divergence found in this study was small, there was evidence of differing regional mitochondrial maternal lines. A survey of chinook populations from California, Oregon, Washington, and other drainages in British Columbia, as well as the use of restriction endonucleases recognizing specific four-base sequences, would further clarify the extent of this geographic structuring. Based on this analysis and the potential for further variation in the southern range, it would appear that this method of analysis does hold promise as a tool for stock identification and fish management.

Acknowledgements

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References

Avise, J. C., C. Giblin-Davidson, J. Laerm, J. C. Patton, and R. A. Lansman. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc. Natl. Acad. Sci. USA 76: 6694–6698.

Berg, W. J., and S. D. Ferris. 1984. Restriction endonuclease analysis of salmonid mitochondrial DNA. Can. J. Fish. Aquat. Sci. 41: 1041–1047.

Brown, W. M., M. George Jr., and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76: 1967–1971.

Giles, R. E., H. Blanc, H. M. Cann, and D. C. Wallace. 1980. Maternal inheritance of human mitochondrial DNA. Proc. Natl. Acad. Sci. USA 77: 6715–6719.

Hale, L. R., and A. T. Beckenbach. 1985. Mitochondrial DNA variation in *Drosophila pseudoobscura* and related species in Pacific Northwest populations. Can. J. Genet. Cytol. 27: 357–364.

Kristiansson, C., and J. D. McIntyre. 1976. Genetic variation in Chinook salmon (*Onchorhynchus tshawytscha*) from the Columbia River and three Oregon coastal rivers. Trans. Am. Fish. Soc. 5: 620–623.

Lansman, R. A., R. O. Shade, J. F. Shapiro, and J. C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. J. Mol. Evol. 17: 214–226.

McPhail, J. D., and C. C. Lindsey. 1970. Freshwater fishes of Northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173: 175–177.

Nei, M., and F. Tajima. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105: 207–217.

Thomas, W. K., R. E. Withler, and A. T. Beckenbach. 1986. Mitochondrial DNA analysis of Pacific salmonid evolution. Can. J. Zool. 64: 1058–1064.

Utter, F. M., D. Campton, S. Grant, G. Milner, J. Seeb, and L. Wishard. 1980. Population structures of indigenous salmonid species of the Pacific Northwest, p. 285–304. *In* W. J. McNeil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press and Oregon State University Grant College Program, Corvallis, OR.

Wilson, G. M., W. K. Thomas, and A. T. Beckenbach. 1985. Intra- and inter-specific mitochondrial DNA sequence divergence in *Salmo*: rainbow, steelhead, and cutthroat trouts. Can. J. Zool. 63: 2088–2094.

Influence of Vegetation and Hydrology on the Humus Budgets of Labrador Lakes[1]

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Engstrom, D. R. 1987. Influence of vegetation and hydrology on the humus budgets of Labrador lakes. Can. J. Fish. Aquat. Sci. 44: 1306–1314.

A mass-balance approach is used to explore the effect of several hydrologic and geographic variables on humic matter concentrations in surface waters of Labrador–Ungava. Water-color measurements from 67 dilute oligotrophic lakes are used to evaluate model predictions of aquatic humus concentration from basin hydrology, catchment vegetation, and internal loss rates. The major trend in water color in the Labrador region — increasing north to south — results from differences in external humus loading from catchment vegetation and soils. Hydrologic influences on aquatic humus concentrations reside in the dilution of catchment drainage by direct precipitation to the lake — effective only when catchments are small — and in water residence time which controls losses due to mineralization and sedimentation.

Une approche par bilan massique a été utilisée pour l'étude des effets de diverses variables hydrologiques et géographiques sur les concentrations de matières humiques des eaux de surface du Labrador et de l'Ungava. La détermination de la couleur de prélèvements d'eau diluée provenant de 67 lacs oligotrophes a servi à évaluer les prévisions d'un modèle de la concentration humique en milieu aquatique à partir de l'hydrologie et de la végétation du bassin versant et des taux de pertes internes. La principale tendance de la couleur de l'eau dans la région du Labrador, qui s'accroît du nord au sud, résulte des écarts de la charge humique externe ayant son origine dans la végétation et les sols du bassin versant. Les facteurs hydrologiques influant sur la concentration des eaux en matières humiques ont trait à la dilution des eaux du bassin versant par les précipitations directes au-dessus des lacs — phénomène efficace pour les petits bassins versants seulement — et le temps de séjour de l'eau qui régit les pertes par minéralisation et sédimentation.

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Highly colored brown-water lakes are a common limnological feature of the boreal landscape worldwide, and although present in other regions, dystrophic or polyhumic waters are most closely associated with northern environments. The coloring in these lakes is caused by high concentrations of dissolved humic compounds (humic and fulvic acids), which are formed by the microbial degradation of plant material, particularly lignin and cellulose. Terrestrial plant and soil systems (including wetlands) are the primary source of aquatic humus in small north-temperate lakes, although decomposition products and exudates of aquatic organisms may be important in some cases.

The central issue in this study is the evaluation of factors contributing to the wide range of humic matter concentrations found in north-temperate lakes. This problem is of limnological importance because aquatic humus is often a major component of dissolved organic carbon (DOC) in oligotrophic lakes and can influence primary productivity through sequestering of phosphorus (e.g. Schindler and Jackson 1975; Stewart and Wetzel 1982) and perhaps iron (Giesy 1976; Jackson and Hecky 1980) or by increased light attenuation and shading (Shapiro 1957). Through complexation, humic compounds may limit the toxicity of other metals such as aluminum (Driscoll et al. 1980, 1984), and as weak acids they can exert a profound influence on pH and acidity of dilute waters (e.g. Brosset 1979; Oliver et al. 1983).

Lakes receiving drainage from peatlands or forests with deep organic soils are typically the most tea-colored (e.g. Rapp et al. 1985), and soil solutions under conifer stands are much richer in DOC than those under hardwoods (Cronan and Aiken 1985), suggesting that the nature of catchment vegetation and soils may be an important factor controlling the concentration of humic compounds in surface waters. Kerekes (1974) suggested that for headwater lakes, color is mainly a function of influent humus concentration, increasing or decreasing in concert with runoff volume. Schindler (1971), on the other hand, proposed that color in lakes may be proportional to the ratio of terrestrial drainage area to lake surface area (A_d/A_o), while Gjessing and Samdal (1968) and Rapp et al. (1985) presented limited data indicating that longer hydraulic residence time may contribute to decreased water color through processes of degradation and sedimentation within the lake.

This study attempts to evaluate further the relationship between water color and several hydrologic and geographic variables in the Labrador–Ungava region of eastern Canada. A mass-balance approach is used to investigate the relative contribution of such factors as vegetation and soils, basin morphometry, climate, and humus degradation rate to the total humus budget of 67 oligotrophic lakes.

Study Area

Labrador forms the easternmost seaboard of continental Canada, extending from 50 to 60°N latitude along the Labrador

[1]Limnological Research Center Contribution No. 336.

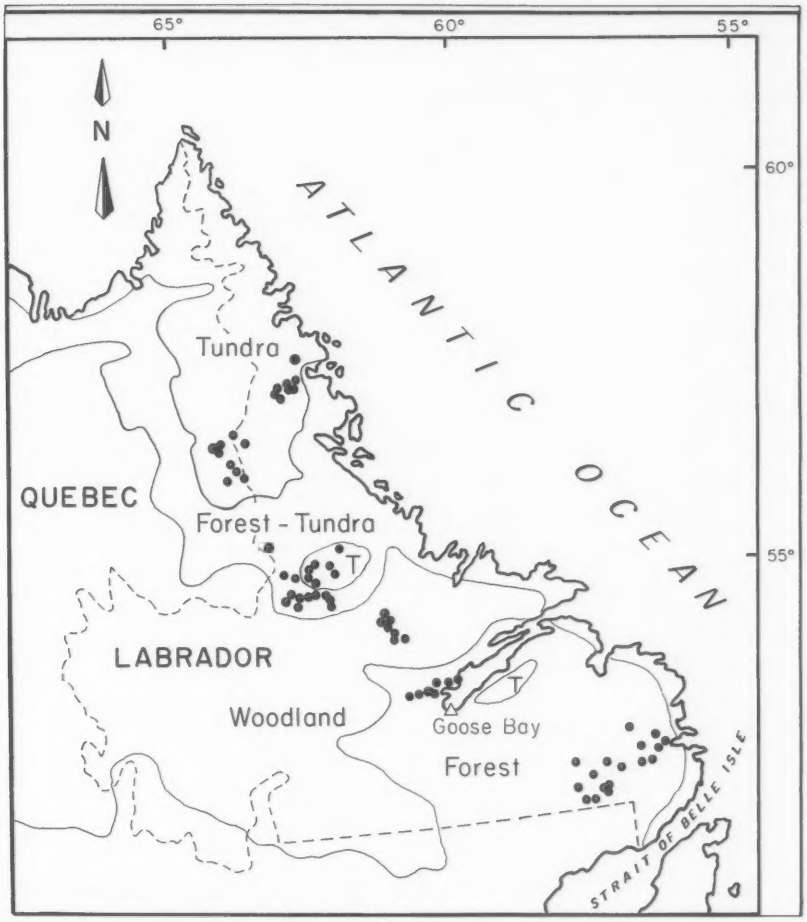

FIG. 1. Location of study lakes in Labrador–Ungava, eastern Canada; vegetation zones according to Hare (1959); T = tundra.

Sea. The area is underlain largely by granitic rocks of the Canadian Shield and is covered throughout by a thin mantle of Wisconsin drift. Labrador's climate is characterized by long cold winters and cool moist summers. Precipitation, which falls equally in summer and winter, ranges from 60 to 110 $cm \cdot yr^{-1}$, increasing north to south.

The vegetation of Labrador may be divided into four broad physiognomic zones (Hare 1959), viz. tundra, forest–tundra, lichen woodland, and boreal forest, which occur roughly along a north–south gradient (Fig. 1). The transition from tundra to forest is widened by rough topography and a southward increase in elevation in central Labrador. Black spruce (*Picea mariana*) is the dominant tree species throughout most of Labrador, although white spruce (*P. glauca*), tamarack (*Larix laricina*), and, in the south, fir (*Abies balsamea*) are locally common. A sparse to dense cover of lichen, heath (Ericaceae), and dwarf willow (*Salix*) predominates in open terrain.

Water chemistry is uniformly dilute throughout most of Labrador, reflecting the granitic nature of the underlying bedrock and till (Clair et al. 1982). In the study lakes, mean alkalinity and conductivity (±SD) were 65 ± 80 $\mu eq \cdot L^{-1}$ and 12 ± 12 $\mu S \cdot cm^{-1}$, respectively, most sulfate concentrations were below 2 $mg \cdot L^{-1}$, and pH values were slightly acid to circumneutral (5.0–7.5).

Methods

A total of 67 lake sites in Labrador and adjacent Quebec were sampled via floatplane during July–August 1979. These sites were clustered in different forested and treeless regions from the Strait of Belle Isle north to Okak Bay, a distance of 800 km (Fig. 1). As part of a more comprehensive water chemistry survey, apparent color as an estimate of humic matter concentration was determined with a field comparator and calibrated platinum (Pt) standards. Water color is a widely employed and reliable proxy for DOC measurement of aquatic humus (Gjessing 1976) and was used in this study to avoid humus losses during lengthy field storage of samples. Catchment and lake surface area were measured on 1 : 50 000 topographic maps and 1 : 17 000 aerial photographs; the latter were also used to characterize predominant catchment vegetation. Nearly all lakes sampled were located in primary watersheds,

TABLE 1. Humus loss rates in water samples from Superior National Forest lakes determined from the regression of water color (O.D. at 350 nm) on storage time under two temperature/light regimes. Regression coefficients (r^2) are shown in parentheses.

Lake	Initial conditions: Color (Pt units)	Initial conditions: Conductivity ($\mu S \cdot cm^{-1}$)	Loss rate (yr^{-1}): Warm/light	Loss rate (yr^{-1}): Cold/dark
Thomas	140	77	0.44 (0.97)	0.04 (0.52)
Christianson	130	29	0.78 (0.89)	0.18 (0.93)
Marble	95	88	0.50 (0.98)	0.09 (0.95)
Lichen	70	40	0.63 (0.78)	0.11 (0.71)
Christine	45	67	0.57 (0.91)	0.18 (0.81)
Gust	50	41	0.54 (0.90)	0.18 (0.84)
Delay	20	61	0.42 (0.55)	0.29 (0.61)
Kane	25	29	0.28 (0.62)	0.19 (0.50)
Dumbell	15	68	0.41 (0.67)	0.19 (0.70)
Divide	15	15	0.24 (0.30)	0.29 (0.74)
Mean			0.48	0.16
SD			0.16	0.08

receiving no significant drainage from large rivers or from other lakes. Lake surface area ranged from 4 to 235 ha (mean = 94 ha).

Laboratory tests were designed to evaluate the rate of humic matter degradation under a range of experimental conditions appropriate for lakes in the Labrador–Ungava region. Duplicate 2-L water samples were obtained from 10 lakes in the Superior National Forest (SNF) of northeastern Minnesota — as a substitute for such samples from Labrador that were logistically unavailable — and incubated under two different light/temperature regimes: warm/light (22°C, 12 h light/dark cycle; cool white illumination at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) and cold/dark (4°C, 24 h dark), for a total of 249 and 454 d, respectively. The rate of humic matter degradation was estimated from periodic measurements of optical density (O.D.) (at 350 nm) on 10-mL aliquots, centrifuged prior to reading. The SNF samples used in this study were from dilute oligotrophic lakes of water chemistry similar to those in Labrador. Initial water color ranged from 15 to 140 Pt units, and sites were roughly paired such that a moderate range of ionic strength (as estimated by conductivity) was provided at each level of apparent color (Table 1).

Results and Discussion

Vegetation and Soils

The concentration of dissolved humus in lake waters of Labrador–Ungava as measured by apparent color is strongly aligned with catchment vegetation (Fig. 2). In northern regions wherever tundra vegetation prevails, water color is invariably low ($\bar{X} \pm SD = 6 \pm 3$ Pt units). Farther south or at lower elevations in spruce woodlands and forest–tundra, lake waters are slightly darker (12 ± 6 Pt units). In the boreal forest zone of southeastern Labrador, most lakes are perceptibly tea-colored, although those with forested uplands are somewhat lighter (50 ± 22 Pt units) than lakes characterized by >25% of open peatlands in their catchments (89 ± 28 Pt units). The differences among these groups are highly significant ($p < 0.05$, B.S.D.) and may be attributed largely to differences in humic acid production by the vegetation and soils of the drainage basin.

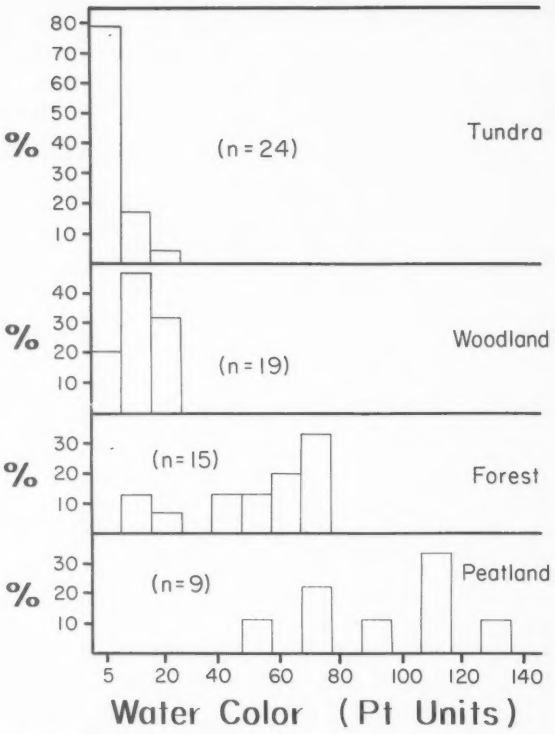

FIG. 2. Frequency distribution of lake water color; sites subdivided according to predominant catchment vegetation.

In southeastern forested catchments, soils are typically histic (peaty) because litter production by coniferous vegetation (particularly the bryophyte ground flora) exceeds decomposition under the regionally humid–subarctic climate. The accumulation of cellulose and lignin in waterlogged acid soils provides a rich medium for humic acid production, while the organic horizon itself inhibits downward percolation of groundwater and the sequestering and degradation of humic materials in deeper soil horizons (cf. Cronan and Aiken 1985). Water movement occurs largely as surface or interflow, and dissolved humus enters streams and lakes without benefit of filtration by the mineral soil. Such conditions occur in extreme on relatively level terrain, where poor drainage has contributed to the accumulation of several metres of peat and the development of sparsely forested mires. Peatland lakes in Labrador, like those worldwide, are characterized by deeply humic-stained waters.

In the more sparsely vegetated woodlands and forest–tundra, soil organic horizons are thin and patchy, lignin and cellulose production is much reduced, and humic acids generated by decomposition are more readily mineralized by percolation of groundwater through the soil profile. As a result, humus loading to surface waters from woodlands is lower than that from forested catchments. On the tundra landscape farther north, lakes are invariably clear because of extremely low vegetational productivity and limited generation of humic compounds from barren tundra inceptisols.

Mass-Balance Model

The relationship between humus concentrations in lakes and the supply of dissolved humic matter from the watershed may be described more explicitly with a mass-balance model analogous to that developed for phosphorus budgets. This exercise is useful because, while differences in production of humic matter among vegetational regions can account for much of the variation in water color observed in Labrador–Ungava, the considerable range in humus concentration exhibited by lakes with apparently similar catchment vegetation may reflect more subtle differences in humus budgets of the land–water system.

The change in lake humus concentration [HA] in a continuously well-mixed system may be simply stated as the sum of gains and losses through inflow, outflow, and sedimentation or degradation:

$$(1) \quad d\mathrm{HA}/dt = J - Q\cdot[\mathrm{HA}] - S$$

where J is the rate of allochthonous humus input, S is the net rate of internal humus loss through sedimentation or mineralization, and Q is the water outflow rate (including groundwater). Under steady-state conditions:

$$(2) \quad [\mathrm{HA}]^* = (J - S)/Q.$$

Because humic compounds are derived largely from terrestrial sources, the annual input J may be defined as the product of the mean concentration of humic matter in runoff $[\bar{h}]$ and the annual inflow of water from the catchment. Catchment inflow, which includes groundwater, may be estimated from excess precipitation ($p - e$; precipitation minus evapotranspiration) and watershed area (A_d), so that

$$(3) \quad J = [\bar{h}]\cdot(p - e)\cdot A_d.$$

Assuming no interaction of regional aquifers (with recharge or discharge outside the catchment), water outflow may be likewise estimated from excess precipitation as

$$(4) \quad Q = (p - e)\cdot(A_d + A_o)$$

where A_o is the lake surface area. To simplify the model, evapotranspiration from the land surface and evaporation from the lake are assumed to be equal. Finally, the internal loss rate (S) may be expressed as a function of humus concentration in the lake:

$$(5) \quad S = F\cdot V$$

where F is the annual decline in humic matter concentration from sedimentation or biochemical oxidation and V is the lake volume. Thus, substituting equations (3), (4), and (5) for J, Q, and S and rearranging terms, equation (2) can be written

$$(6) \quad [\mathrm{HA}]^* = \frac{A_d\cdot[\bar{h}]}{(A_d + A_o)} - \frac{F\cdot V}{(A_d + A_o)\cdot(p - e)}.$$

If the internal loss rate is zero or negligible, equation (6) reduces to

$$(7) \quad [\mathrm{HA}]^* = \frac{A_d\cdot[\bar{h}]}{(A_d + A_o)}$$

which is to say that lake humus concentration (neglecting sedimentation or mineralization) is simply equivalent to the mean concentration of humic matter in runoff from the catchment normalized to the ratio of catchment area (whence humic compounds are derived) to the total watershed area (lake plus catchment). This relationship states that, all other factors being equal, humus concentration in a lake with a large catchment (relative to lake surface area) will be higher than in a lake with a small catchment because of dilution of humic matter in runoff by direct precipitation to the lake surface.

Furthermore, if the loss rate (F) is assumed to be a constant proportion of the mean concentration of the lake prior to degradation or sedimentation, i.e.

$$(8) \quad F = K\cdot\frac{A_d\cdot[\bar{h}]}{(A_d + A_o)}$$

where the constant K is the fractional decrease in humus concentration within the lake, and $\bar{Z}\cdot A_o$ (mean depth times lake surface area) is substituted for lake volume, equation (6) becomes

$$(9) \quad [\mathrm{HA}]^* = \frac{A_d/A_o\cdot[\bar{h}]}{(A_d/A_o + 1)}\cdot\left\{1 - \frac{\bar{Z}\cdot K}{(A_d/A_o + 1)\cdot(p - e)}\right\}.$$

The terms in equation (9) become readily transparent when compared with those in the standard phosphorus model of Vollenweider (1975):

$$(10) \quad [P]^* = [\bar{\mathrm{p}}]\cdot(1 - R_\mathrm{p}).$$

The mean influent phosphorus concentration, $[\bar{\mathrm{p}}]$, is equivalent to $(A_d/A_o\cdot[\bar{h}])/(A_d/A_o + 1)$ and the phosphorus retention coefficient R_P is analogous to the loss function from (9):

$$(11) \quad -\frac{\bar{Z}\cdot K}{(A_d/A_o + 1)\cdot(p - e)}.$$

Equation (11) reduces to $K\cdot\tau_w$ where τ_w is the hydraulic retention time in years. In this form, internal loss of humic matter is accounted for by lake volume normalized to water inflow and the loss constant (K). Restated in qualitative terms, the longer humic compounds reside in the lake, the larger is the fraction lost to the sediments or to mineralization. An analogous construction of R_p in terms of hydraulic retention time (or its inverse, the hydraulic flushing rate) provides the best fit for empirical values of phosphorus retention in oligotrophic lakes (Larsen and Mercier 1976).

Humus Loss Rates

The determination of internal loss of humic matter is critical to the prediction of lake humus concentrations from the mass-balance equation outlined above. However, empirical estimation of humus loss rates from lake inflow and outflow data is usually impossible because the input term is unknown for most lakes. An alternative approach utilized in this study is the laboratory assessment of humus loss rates under conditions where both inflow and outflow are eliminated and where decreasing concentrations over time may be ascribed solely to sedimentation and mineralization of dissolved humic matter.

Although laboratory experiments are often a poor representation of field conditions, several aspects of humus degradation implicit in the mass-balance loss function may be evaluated by experimental data: (1) A primary assumption of the model as stated in equation (9) is that internal loss is proportional to the concentration of humic matter, i.e. the internal loss coefficient (K) is constant over time within a lake. Under laboratory conditions with no inputs or outflow, humus loss should be a linear function of time. (2) The only independent factor other than K in the loss function is the hydraulic retention time. Under a similar hydraulic regime, loss rates should be the same for water samples from different lakes and independent of the

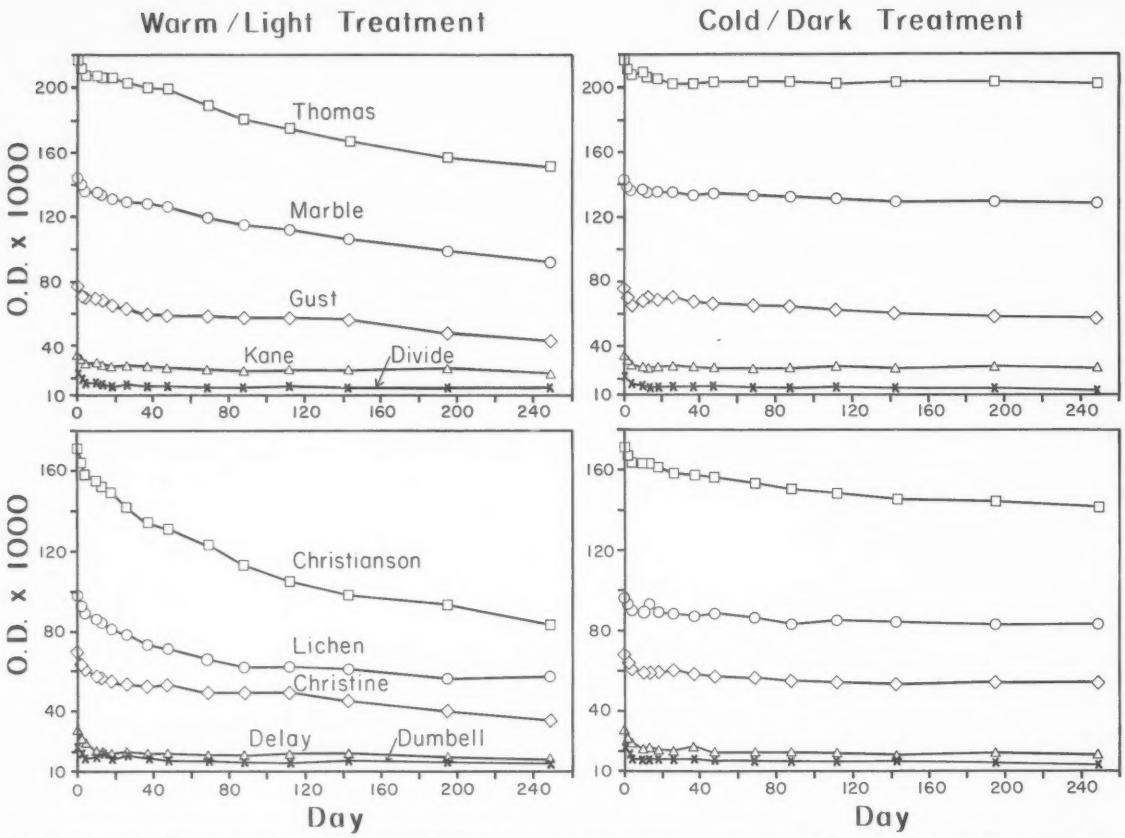

FIG. 3. Decrease in humus concentration (as measured by O.D. at 350 nm) in water samples from 10 Superior National Forest lakes during storage under warm/light (22°C, 12 h light/dark) and cold/dark (4°C, 24 h dark) conditions.

initial concentration of humic matter or other aspects of water chemistry. (3) An order-of-magnitude assessment of humus stability is needed to ascertain whether in situ loss rates are so low as to be insignificant or so high as to dominate mass-balance considerations.

Results from the long-term incubation of water samples taken from 10 lakes in the SNF of Minnesota provide this needed estimate of humus degradation rates and their variability. The change in humus concentration in water samples as measured by O.D. (350 nm) is plotted versus time in Fig. 3. Most lake waters under both warm and cold experimental conditions exhibit a linear decline in O.D. over the long term. However, in all cases a break in slope is evident in measurements taken after 4 d. The higher loss rate measured immediately following collection is attributed to the rapid decay or sedimentation of nonhumic labile organics which are presumably supplied to the lake from internal sources such as mineralization of seston. These labile materials are quickly exhausted, and a gradual absorbance decline ensues with the slow decay of the more refractory humic components.

Accepting only the long-term decline as humus degradation, loss rates can be calculated from the regression of O.D. on time after day 4 (Table 1). Although a few loss profiles appear to be first-order (e.g. Christianson and Lichen lakes under warm/light treatment; Fig. 3), linear regression coefficients are sufficiently high in most cases to justify treating the loss coefficient (K) as a constant for an individual lake. This conclusion, however, presupposes a steady-state temperature and light regime. Humus loss rates under warm/light experimental conditions are significantly greater than those under a cold/dark treatment, presumably because of greater biochemical activity or photooxidation in the former (Fig. 3; Table 1). While this difference indicates the importance of temperature and light conditions on humus degradation, these factors may be assumed to be reasonably constant over the long term within a single lake or in a series of lakes under a similar regional climate.

The measured loss rates for lake samples within treatment groups are sufficiently similar to justify the second assumption that the loss coefficient (K) may be treated as a regional constant ($K \pm \text{SD} = 0.48 \pm 0.16$ and $0.16 \pm 0.08 \cdot \text{yr}^{-1}$ for warm and cold treatments, respectively; Table 1). Additional support for this contention is provided by multiple regression of loss rates on temperature/light regime (as a categorical weight), initial humus concentration, and conductivity (as a measure of water chemistry). These relations indicate that the latter two variables are not a significant influence on humus loss rates ($p > 0.5$; $t = 0.61, -0.47$, respectively). Moreover, the mean loss rates observed here are very similar to those reported by Gjessing (1976) for reduction in DOC during storage of aquatic

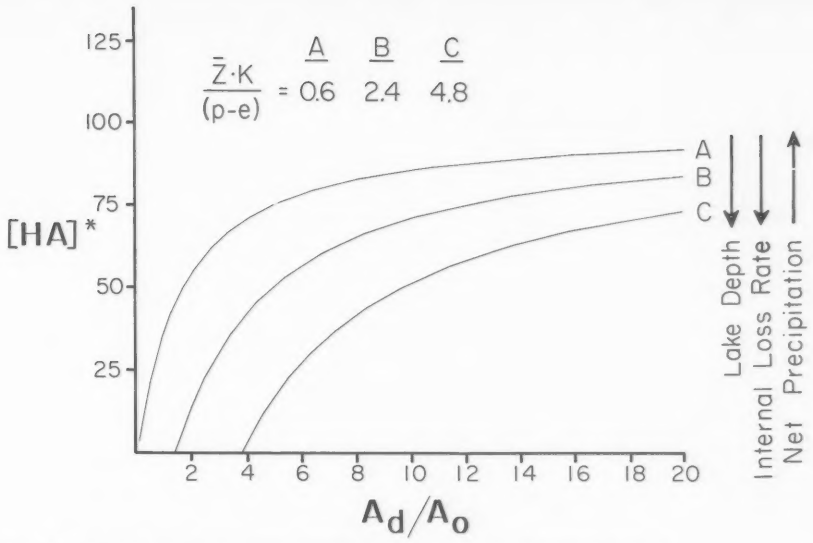

FIG. 4. Predicted lake humus concentration [HA]* from the mass balance equation

$$[HA]^* = \frac{A_d/A_o \cdot [\bar{h}]}{(A_d/A_o + 1)} \cdot \left\{1 - \frac{\bar{Z}\cdot K}{(A_d/A_o + 1)\cdot(p - e)}\right\}$$

where A_d/A_o = catchment area/lake surface area, $[\bar{h}]$ = mean influent humus concentration, $\bar{Z}$ = mean lake depth, K = loss rate constant, and $(p - e)$ = net precipitation. The family of curves (A, B, and C) were generated from a range of values for Labrador such that $\bar{Z}\cdot K/(p - e) = 0.6$, 2.4, and 4.8, respectively; $[\bar{h}] = 100$ for all curves. A trend from A to C results from increasing lake depth or loss rate, or decreasing net precipitation.

humus samples from Norway: $0.52 \cdot yr^{-1}$ at room temperature (light conditions unspecified) and $0.09 \cdot yr^{-1}$ during cold (4°C) dark storage.

The question as to what constitutes a reasonable estimate for loss rates in situ is more difficult to answer. The temperature/light treatments selected for this study are extremes unlikely to be met by field conditions in Labrador–Ungava. Lakes in this region are frozen for 7–9 mo of the year, and throughout most of the open-water season, epilimnetic temperatures do not exceed 10°C. Thus, loss rates measured under the warm/light experimental regime (22°C, 12 h light/dark) can be viewed as an upper limit (excluding the unknown contribution of natural UV light). More likely, humus degradation rates approach those measured under the cold/dark regime (4°C, 24 h dark), which better approximates lake conditions in winter and the hypolimnion year-round. Values for the loss constant (K) of 0.05, 0.2, and $0.4 \cdot yr^{-1}$ are used in the evaluation of the mass-balance model in the following section.

Evaluation of the Model

In order to explore the predictions of the humus mass-balance model, boundary conditions for the parameters in equation (9) were set according to a range of values appropriate for the Labrador study region. Estimated net annual precipitation ($p - e$) in Labrador ranges from 0.6 m in the southeast to 0.4 m in the north on the basis of northward latitudinal gradients in total precipitation of $1.0–0.7 \text{ m} \cdot yr^{-1}$ (Peach 1975) and open-water evaporation of $0.4–0.3 \text{ m} \cdot yr^{-1}$ (Ferguson et al. 1970). Potential evapotranspiration estimates for Labrador of approximately $0.4 \text{ m} \cdot yr^{-1}$ (Rowe 1972) are comparable with the open-lake evaporation measurements, which justifies the use of a single term for evaporation and evapotranspiration in simplifying equation (9) above. Detailed bathymetric profiles were obtained for only a few of the 67 study lakes, so that mean lake depth ($\bar{Z}$) had to be inferred from maximum depth measurements (Z_{max}). From the empirical relationship $\bar{Z} = 0.45Z_{max}$ (derived from those lakes where mean depth was determined) and an average maximum depth (±SD) of 13.4 ± 9.9 m, values of 6 and 1.5–12 m were chosen for average and extreme values for mean depth. Influent concentrations of humic matter measured on selected inlet streams ranged from near zero to 150 Pt units, and catchment/lake area ratios for the 67 lakes varied from 0.5 to 20. These values were used to establish limits for $[\bar{h}]$ and A_d/A_o, respectively, while the humus loss constant (K) was estimated according to experimental results obtained above.

A series of curves depicting equilibrium humus concentration [HA]* as a function of catchment/lake area ratios (A_d/A_o) are shown in Fig. 4. Each curve represents the predicted concentration of humic matter in a lake with a mean influent concentration $[\bar{h}]$ of 100 Pt units under a set of hydrologic conditions resulting in a given value for the loss term, $\bar{Z}\cdot K/(p - e)$. Curve B, for example, represents what are estimated here to be average Labrador conditions of mean lake depth ($\bar{Z} = 6$ m), loss constant ($K = 0.2 \cdot yr^{-1}$), and net precipitation ($p - e = 0.5 \text{ m} \cdot yr^{-1}$), such that in equation (9), $\bar{Z}\cdot K/(p - e) = 2.4$. Curve C is obtained either by decreasing net precipitation or increasing mean depth or the internal loss constant such that $\bar{Z}\cdot K/(p - e) = 4.8$. Such conditions represent

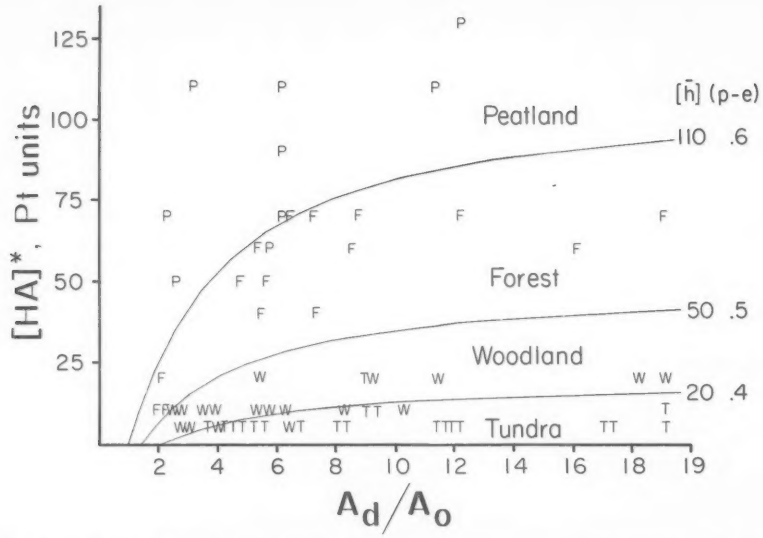

FIG. 5. Relation of water color in Labrador lakes (as a measure of humus concentration [HA]*) to the ratio of A_d/A_o; lakes classified according to predominant catchment vegetation. The curves delineating lake groups were generated from the mass-balance equation defined in Fig. 4, with $\bar{Z}$ = 6 m, K = 0.2·yr^{-1}, and values for $[\bar{h}]$ and $(p - e)$ as indicated.

a much higher rate of humus loss than curve B and would result if $\bar{Z}$ or K were independently doubled or $(p - e)$ were halved. Conversely, curve A depicts humus concentrations under a much lower value for the loss term, $\bar{Z}\cdot K/(p - e) = 0.6$, derived from an independent increase in $(p - e)$ or a decrease in $\bar{Z}$ or K by a factor of 4. In essence, curves A and C depict values of the loss term from equation (9) that would occur at maximum and minimum values (estimated for Labrador) of any of three independent variables (net precipitation, mean depth, and loss rate). More extreme values of the loss term could arise if, for example, $(p - e)$ were increased and K and $\bar{Z}$ decreased simultaneously. However, the range of conditions displayed in Fig. 4 serves to depict reasonable values of lake humus concentrations and reveals the significant predictions of the model.

It is apparent from the series of curves in Fig. 4 that predicted humus concentration, i.e. water color, increases asymptotically toward the influent concentration, $[\bar{h}]$, at high values of A_d/A_o and that water color is relatively independent of the catchment/lake area ratio for lakes with large catchments. In curve B, for example, [HA]* is highly dependent on A_d/A_o below a value of 6 and largely unaffected by changes in A_d/A_o above a value of 10. This pattern results primarily from the effects of dilution by direct precipitation to the lake surface, which is only important for lakes with very small catchments.

Another explicit prediction of the model is that for a given value of A_d/A_o and with other factors constant, water color will be lower in deep lakes, in lakes with a high rate of humus degradation, or in regions with lower net precipitation. These three factors control internal loss of humic matter, which is relatively unimportant in curve A, while it is fairly significant in the total humus budget in curve C.

The influence of the above parameters on water color is less for lakes with large watersheds because these have high flushing rates, which lower internal loss (retention) and hence the difference between the curves. At an A_d/A_o ratio of 5, for example, the difference in humus concentration between curves is large ([HA]* = 75, 50, and 16 for A, B, and C, respectively), while at A_d/A_o = 20 the values for [HA]* are more similar (93, 84, and 73).

Certain predictions of the mass-balance model may be tested with empirical data gathered from the 67 study lakes in Labrador. The surface area of lakes and their catchments is measurable from topographic maps, while the single determination of water color at each site can be used as an estimate of equilibrium humus concentration — although with caution, as considerable seasonal variation in water color can occur in some lakes (e.g. Tipping and Woof 1983; Visser 1984). Mean lake depth and humus loss rates are not explicitly known for each site, so it is not possible to evaluate the accuracy of the internal loss term as formulated in equation (9). Nevertheless, a plot of measured water color versus A_d/A_o together with theoretical curves from the mass-balance model (Fig. 5) is instructive. In this diagram, lake sites, which have been identified as to predominant catchment vegetation as peatland, forest, lichen woodland (including forest–tundra), and tundra, are delineated into distinct groupings by three curves generated from equation (9). The lines are drawn using estimated mean values for $\bar{Z}$ (6 m) and K (0.2·yr^{-1}), the latitudinal gradient in net precipitation, and fitted (but reasonable) values for $[\bar{h}]$, the mean influent concentration.

Two important points may be gained from this exercise. First, the Labrador lake data broadly support the asymptotic prediction of the model. In particular, lakes with low color in forest and peatland regions have generally low A_d/A_o ratios (the small variation in water color among forest–tundra and tundra lakes precludes a similar conclusion for these groups). Thus the very low water color for certain forested lakes may be explained by dilution of humus inputs from their relatively small catchments by direct precipitation to the lake surface. Conversely, the lack of relationship between [HA]* and rela-

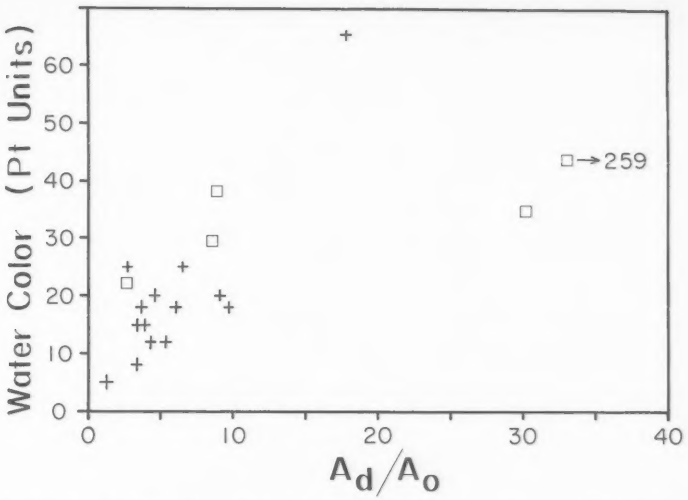

FIG. 6. Relation of water color to the ratio A_d/A_o for 14 lakes in northwestern Ontario (+) (Schindler 1971) and 5 lakes in Newfoundland (□) (Kerekes 1974).

tive catchment size at high values for A_d/A_o is consistent with the model's prediction that the influence of catchment size is only felt at low A_d/A_o. Additional variation within groups may be due to individual lake differences in $\bar{Z}$, K, or $[\bar{h}]$ that cannot be evaluated with available data.

The second conclusion drawn from Fig. 5 is that major trends in water color on a regional basis result primarily from external loading of humic matter, which as illustrated here is a function of vegetation (and associated soils). If the model's predictions were taken literally, it would be concluded that mean influent concentration of aquatic humus in tundra lakes is less than 20 Pt units, in forest lakes 50−110 Pt units, and so on. These predictions could be tested with more detailed measurements of humus concentrations in streams and groundwater in the different vegetational regions.

Conclusions

From the theoretical and empirical analyses presented in this study, vegetation and hydrology emerge as important factors controlling the humus content (and hence color) of lake waters in eastern Canada. The vegetation−soil system controls the primary flux of aquatic humus to streams and lakes through its influence on both the production and sequestering of humic compounds. Deep organic horizons, which tend to develop under closed-canopy conifer forests, particularly in humid and poorly drained landscapes, serve not only as a rich source of humic substances but also inhibit downward percolation and sequestering of these organic acids in deeper mineral horizons.

The importance of internal soil drainage in controlling humus flux is further illustrated by limnological conditions found in recently deglaciated areas (<300 yr old) of Glacier Bay, Alaska. Insufficient time has elapsed for deep organic layers or placic soil horizons to develop beneath the forests growing on this young landscape (Ugolini and Mann 1979). As a result, Neoglacial lakes have exclusively clear water whereas outside the limits of recent glaciation, paludified soils inhibit internal soil drainage and highly colored streams and lakes predominate (D. R. Engstrom, unpubl. data).

Hydrologic influences on lake humus concentrations reside both in the dilution of catchment drainage by direct precipitation to the lake — effective only when catchments are relatively small — and in controlling water residence time and hence internal losses due to degradation and sedimentation. The asymptotic predictions of the mass-balance model are further supported by empirical data reported by Kerekes (1974) for Newfoundland and Schindler (1971) for the Experimental Lakes Area of Ontario. Kerekes found little relationship between average water color and the ratio A_d/A_o except for sites where $A_d/A_o < 10$. Schindler, on the other hand, concluded that water color was directly related to relative catchment size. However, for nearly all of the lakes in his data set, $A_d/A_o < 10$. Thus the prediction that water color will be influenced by catchment size only at small A_d/A_o is affirmed by the combined results of these two studies (Fig. 6).

The relative magnitude of internal humus loss (retention) is difficult to assess with the available data. However, several general predictions derived from the mass-balance model may be useful in future testing. In Labrador the internal loss term is probably unimportant for most lakes because of high flushing rates and presumably low degradation/sedimentation rates. Internal loss should be more important in lake budgets for regions with lower net precipitation and hence longer hydraulic residence times, in warmer climates with a longer ice-free season and predictably higher loss rates, and potentially in lakes with much higher concentrations of polyvalent cations such as Al and Ca, which can contribute to more rapid polymerization and precipitation of colloidal humates (Almer et al. 1978; Tipping and Ohnstad 1984; Effler et al. 1985). Loss rates in this study were assumed from laboratory experiments to be relatively constant among sites. However, because of the uniformly dilute nature of the lakes, the influence of other aspects of water chemistry on precipitation and degradation of aquatic humus needs further evaluation.

Acknowledgments

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References

ALMER, B., W. DICKSON, C. EKSTROM, AND E. HORNSTROM. 1978. Sulfur pollution and the aquatic ecosystem, p. 271–311. *In* J. O. Nriagu [ed.] Sulfur in the environment: Part II, ecological impacts. John Wiley & Sons, New York, NY.

BROSSET, C. 1979. Factors influencing pH in lake water. Water Air Soil Pollut. 11: 57–61.

CLAIR, T. A., D. R. ENGSTROM, AND W. WHITMAN. 1982. Water quality of the surface waters of Newfoundland and Labrador. Environment Canada Data Report IWD-AR-WQB-82-83, Inland Waters Directorate, Moncton, N.B. 55 p.

CRONAN, C. S., AND G. R. AIKEN. 1985. Chemistry and transport of soluble humic substances in forested watersheds of the Adirondack Park, New York. Geochim. Cosmochim. Acta 49: 1697–1705.

DRISCOLL, C. T., J. P. BAKER, J. J. BISOGNI, AND C. L. SCHOFIELD. 1980. Effects of aluminium speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

1984. Aluminum speciation and equilibria in dilute acidic surface waters of the Adirondack region of New York State, p. 55–75. *In* O. P. Bricker [ed.] Geological aspects of acid deposition. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

EFFLER, S. W., G. C. SCHAFRAN, AND C. T. DRISCOLL. 1985. Partitioning light attenuation in an acidic lake. Can. J. Fish. Aquat. Sci. 42: 1707–1711.

FERGUSON, H. L., A. D. J. O'NEILL, AND H. F. CORK. 1970. Mean evaporation over Canada. Water Resour. Res. 6: 1618–1633.

GIESY, J. P. 1976. Stimulation of growth in *Scenedesmus obliquus* (Chlorophyceae) by humic acids under iron limited conditions. J. Phycol. 12: 172–179.

GJESSING, E. T. 1976. Physical and chemical characteristics of aquatic humus. Ann Arbor Science Publishers Inc., Ann Arbor, MI. 120 p.

GJESSING, E. T., AND J. E. SAMDAL. 1968. Humic substances in water and the effect of impoundment. J. Am. Water Works Assoc. 60: 451–458.

HARE, F. K. 1959. A photo-reconnaissance of Labrador–Ungava. Geographical Branch, Canada Department of Mines and Technical Resources, Memoir 6.

JACKSON, T. A., AND R. E. HECKY. 1980. Depression of primary productivity by humic matter in lake and reservoir waters of the boreal forest zone. Can. J. Fish. Aquat. Sci. 37: 2300–2317.

KEREKES, J. J. 1974. Limnological conditions in five small oligotrophic lakes in Terra Nova National Park, Newfoundland. J. Fish. Res. Board Can. 31: 555–583.

LARSEN, D. P., AND H. T. MERCIER. 1976. Phosphorus retention capacity of lakes. J. Fish. Res. Board Can. 33: 1742–1750.

OLIVER, B. G., E. M. THURMAN, AND R. L. MALCOLM. 1983. The contribution of humic substances to the acidity of colored natural waters. Geochim. Cosmochim. Acta 47: 2031–2035.

PEACH, J. A. 1975. The tourism and recreation climate of Newfoundland and Labrador. Department of Environment Canada, Atmospheric Environment Service, REC-2-75.

RAPP, G. JR., J. D. ALLERT, B. W. LIUKKONEN, J. A. ILSE, O. L. LOUCKS, AND G. E. GLASS. 1985. Acid deposition and watershed characteristics in relation to lake chemistry in northeastern Minnesota. Environ. Int. 11: 425–440.

ROWE, J. S. 1972. Forest regions of Canada. Canadian Forest Service, Publication 1300: 172 p.

SCHINDLER, D. W. 1971. A hypothesis to explain differences and similarities among lakes in the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28: 295–301.

SCHINDLER, D. W., AND T. A. JACKSON. 1975. The biogeochemistry of phosphorus in an experimental lake environment: evidence for the formation of humic–metal–phosphate complexes. Verh. Int. Ver. Limnol. 19: 211–221.

SHAPIRO, J. 1957. Chemical and biological studies of yellow organic acids of lake water. Limnol. Oceanogr. 2: 161–179.

STEWART, A. J., AND R. G. WETZEL. 1982. Influence of dissolved humic materials on carbon assimilation and alkaline phosphatase activity in natural algal–bacterial assemblages. Freshwater Biol. 12: 369–380.

TIPPING, E., and M. OHNSTAD. 1984. Aggregation of aquatic humic substances. Chem. Geol. 44: 349–357.

TIPPING, E., AND C. WOOF. 1983. Seasonal variations in the concentrations of humic substances in a soft-water lake. Limnol. Oceanogr. 28: 168–172.

UGOLINI, F. C., AND D. H. MANN. 1979. Biopedological origin of peatlands in southeast Alaska. Nature (Lond.) 281: 366–368.

VISSER, S. A. 1984. Seasonal changes in the concentration and color of humic substances in some aquatic environments. Freshwater Biol. 14: 79–87.

VOLLENWEIDER, R. A. 1975. Input–output models with special reference to the phosphorus loading concept in limnology. Schweiz. Z. Hydrol. 37: 53–83.

Frequency of Sampling Required for Monitoring the Niagara River

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Water samples have been collected routinely at the head and mouth of the Niagara River since 1983 and analyzed for their chemical and physical characteristics. Statistical techniques are given for estimating the (1) frequency of sampling the river at two locations within a given year and (2) number of years required for monitoring the river in order to detect a linear trend in its water quality.

Depuis 1983, des échantillons d'eau ont été régulièrement prélevés à la source et à l'embouchure de la rivière Niagara et leurs caractéristiques chimiques et physiques ont été déterminées. L'auteur présente des techniques statistiques pour l'estimation 1) de la fréquence de l'échantillonnage de l'eau en deux points au cours d'une année donnée et 2) du nombre d'années de contrôle nécessaire pour déceler une tendance linéaire de la qualité de l'eau.

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Environment Canada has been routinely measuring the physical and chemical characteristics of water samples collected at the head and mouth of the Niagara River (Fig. 1) since 1983. The basic objectives are to use the generated data to make inferences about the differences between the quality of waters at the head and mouth of the river and to estimate the additional load to the river along its course. The design used to collect the samples and the characteristics of the generated data show that (1) water samples were collected at Niagara on the Lake (NOTL) and Fort Erie (FE) on the same day and (2) the concentrations, especially of contaminants in many water samples, are below the level of detection.

Because of (1), a paired comparison statistical test is the most natural approach for evaluating the significance of the difference in the concentration between the two locations, since such a test is not influenced by the variabilities of the concentration from day to day. Also, because of (2), parametric tests such as a matched paired *t*-test are very difficult to compute and interpret, and nonparametric tests which require the calculation of ranks such as Wilcoxon signed rank test are not possible to evaluate due to difficulties in assigning ranks to the differences among the concentrations. It should be stated that if a portion of the data at each of the two stations is below the level of detection, then the sign test is the only exact test available for assessing the differences between NOTL and FE.

The aims of this paper are twofold. The first is to provide a technique for estimating the necessary frequency of taking paired samples from the two locations for monitoring the river within a 1-yr period. The second is to estimate the number of years required for monitoring the river in order to detect a linear trend in the water quality of the river. This is done by assuming that the sign test will be used to detect differences between the two locations. The choice of the sign test is based on the following three facts: (1) values below detection are likely to occur when measuring the concentration of contaminants in water samples and the sign test is the only exact test available that deals with this situation; (2) the sign test is very simple to apply and requires very few assumptions; and (3) the estimated sample size based on the sign test is conservative in the sense that the more efficient parametric and nonparametric tests will require smaller sample size than that required to achieve the same efficiency by the sign test.

Sign Test

Let y_i and x_i be the concentrations on day i, $i = 1, 2, \ldots, N$, at NOTL and FE, respectively. The null hypothesis H_0 states that the observed differences

$$d_i = y_i - x_i \qquad (i = 1, 2, \ldots, N)$$

are the results of chance. In the calculation of the sign test, the random variable Z_i is defined by

$$Z_i = \begin{cases} 1 & \text{if } d_i > 0 \\ 0 & \text{if } d_i < 0 \end{cases}$$

and the null hypothesis is

$$H_0\text{: Prob } \{Z_i = 1\} = \text{Prob } \{Z_i = 0\} = 0.5.$$

The data needed for testing H_0 can be represented in the following contingency table:

		NOTL	
	Concentration	Below detection	Above detection
FE	Below detection	N_{00}	N_{01}
	Above detection	N_{10}	N_{11}

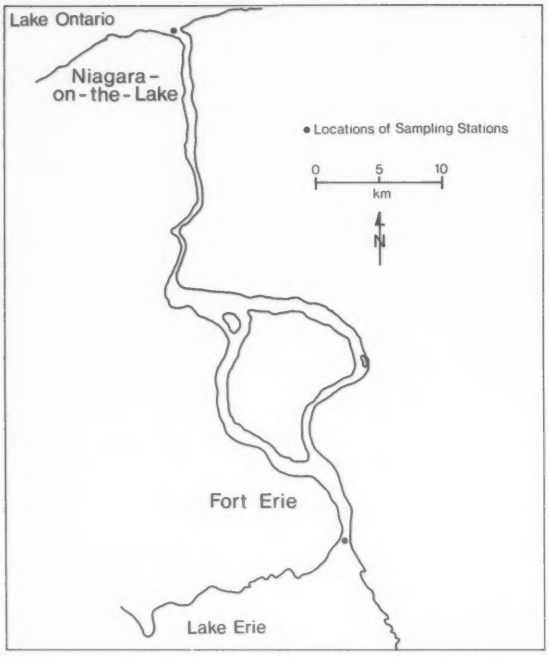

FIG. 1. Location of sampling stations on the Niagara River.

where N_{00} = the number of days in which both y_i and x_i are below the detection limit, N_{01} = the number of days with y_i above detection and x_i below detection, N_{10} = the number of days with y_i below detection and x_i above detection, and N_{11} = the number of days with both y_i and x_i are above the level of detection. In the application of the sign test, N_{00} is ignored and the test is conducted conditionally on

$$N = N_{01} + N_{10} + N_{11}.$$

Suppose that for r_0 days out of the N days, the concentration at NOTL exceeds that at FE; then the exact significance level α is given as

$$\alpha = 2^{-N} \sum_{r=r_0}^{N} {}_NC_r$$

where ${}_NC_r = N!/[r!(N-r)!]$. If α is below a prespecified value (say 0.05), then H_0 is rejected at the significance level α.

Note that α is determined assuming a one-sided test; however, this is not necessary for using the methods of this paper, since the corresponding calculations in the two-sided case can be performed in an obvious manner.

Estimation of the Number of Samples When the Sign Test Is Used

In testing H_0, N_{00} was ignored and only N was used. This is reasonable after the data have been collected, but in sample size estimation, N_{00} cannot be ignored, since it is not known before the data collection. Let M be the number of days that need to be sampled, N the number of usable pairs in the calculation of the sign test, and r the number of pairs for which $y_i > x_i$. Then the conditional distribution of r given N is

$$\text{Prob}\,(r|N) = {}_NC_r\theta^r(1-\theta)^{N-r}$$

where θ is the Prob $\{Z_i = 1\}$. Further, let P_{00} be the probability that y_i and x_i are below the level of detection. Then

$$\begin{aligned}\text{Prob}\,(N_{00}|M) &= {}_MC_{N_{00}}P_{00}^{N_{00}}(1-P_{00})^{M-N_{00}} \\ &= {}_MC_NP_{00}^{M-N}(1-P_{00})^N.\end{aligned}$$

Therefore

$$\begin{aligned}\text{Prob}\,(r|M) &= \sum_N \text{Prob}\,(r|N)\,\text{Prob}\,(N|M) \\ &= {}_MC_r\{(1-P_{00})\theta\}^r\{1-(1-P_{00})\theta\}^{M-r}\end{aligned}$$

Once Prob $(r|M)$ is derived, it is then possible to estimate M. To do this, it is important to distinguish between two types of errors which are associated with any statistical test. Type I is estimated by the probability α of rejecting H_0 when it is true. This probability is commonly known as the significance level. Type II represents the risk of accepting H_0 when H_1 is true. Let β be the probability of committing the second type of error; $(1-\beta)$ is known as the power of the test.

From the above, α and β are given by

$$\alpha = 2^{-M}\sum_{r=r_0}^{M} {}_MC_r(1-P_{00})^r(1+P_{00})^{M-r}$$

$$\beta = \sum_{r=0}^{r_0-1} {}_MC_r\{(1-P_{00})\theta\}^r\{1-(1-P_{00})\theta\}^{M-r}.$$

M can be determined by solving the above two equations for specified values for α, β, θ, and P_{00}. If the binomial distribution is approximated by the normal distribution, then M is given by

$$M = \frac{[\Phi^{-1}(\beta)\sqrt{\theta\{1-(1-P_{00})\theta\}} - 0.5\,\Phi^{-1}(1-\alpha)\sqrt{1+P_{00}}]^2}{(1-P_{00})(0.5-\theta)^2}.$$

Further, taking $\alpha = \beta$, we get

$$M = \frac{\{\Phi^{-1}(1-\alpha)\}^2[2\sqrt{\theta\{1-(1-P_{00})\theta\}} + \sqrt{1+P_{00}}]^2}{(1-P_{00})(1-2\theta)^2}$$

where

$$\Phi(x) = \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}} e^{-x^2/2}\,dx$$

and $\Phi^{-1}(x)$ is the inverse of $\Phi(x)$.

Applications

The previous results are used to estimate the minimum number of paired samples M needed from NOTL and FE when $\alpha = \beta = 0.05$. Table 1 gives the values of M as a function of

TABLE 1. Estimated number of paired samples M for monitoring the head and mouth of the Niagara River.

	P_{00}					
θ	0.0	0.1	0.2	0.3	0.4	0.5
0.500	∞	∞	∞	∞	∞	∞
0.510	27054.837679	33128.338130	40720.160620	50481.028809	63495.477325	81715.664679
0.520	6759.649367	8293.046292	10209.737473	12674.006834	15959.655782	20559.522799
0.530	3001.279955	3689.466837	4549.643479	5655.535522	7130.013552	9194.240326
0.540	1685.849231	2076.712709	2565.233022	3193.279679	4030.629335	5202.875408
0.550	1076.991151	1329.552056	1645.191986	2050.962277	2591.942216	3349.269689
0.560	746.251749	923.316701	1144.584421	1429.017368	1808.212921	2339.041232
0.570	546.824559	678.147003	842.234212	1053.147649	1334.315893	1727.904929
0.580	417.346812	518.878159	645.674004	808.639629	1025.876122	1329.959644
0.590	328.642829	409.585039	510.691846	640.626868	813.821182	1056.244565
0.600	265.162835	331.337455	413.982027	520.177975	661.718776	859.826126
0.610	218.192820	273.390523	342.311057	430.859825	548.869592	714.032357
0.620	182.466222	229.276980	287.711789	362.774552	462.802808	602.790294
0.630	154.660426	194.914846	245.150021	309.670527	395.639554	515.942928
0.640	132.595225	167.624423	211.324683	267.440097	342.202622	446.814539
0.650	114.791938	145.587446	183.992012	233.297140	298.975740	390.870046
0.660	100.218986	127.534859	161.586092	205.291628	263.502396	344.940456
0.670	88.138953	112.559088	142.986631	182.030578	234.024126	306.756767
0.680	78.013377	99.997128	127.374927	162.495099	209.255120	274.659476
0.690	69.441642	89.355492	114.141383	145.926300	188.237423	247.411942
0.700	62.120698	80.260720	102.824499	131.749481	170.245399	224.077214
0.710	55.817874	72.425963	93.069679	119.522931	154.721180	203.934805
0.720	50.352130	65.627940	84.600808	108.902597	141.230126	186.423249
0.730	45.580892	59.690745	77.200283	99.617252	129.429505	171.099710
0.740	41.390637	54.474272	70.694723	91.450723	119.046087	157.611072
0.750	37.690066	49.865805	64.944600	84.228995	109.859867	145.672945
0.760	34.405072	45.773834	59.836597	77.810699	101.692071	135.054184
0.770	31.474987	42.123434	55.277907	72.080022	94.396204	125.565336
0.780	28.849758	38.852784	51.191933	66.941348	87.851287	117.049917
0.790	26.487777	35.910503	47.514996	62.315169	81.956698	109.377739
0.800	24.34225	33.253604	44.193806	58.134926	76.628182	102.439772
0.810	22.419766	30.845895	41.183489	54.344559	71.794755	96.144137
0.820	20.659535	28.656725	38.446038	40.896574	67.396268	90.412961
0.830	19.052324	26.659997	35.949089	47.750517	63.381471	85.179882
0.840	17.579941	24.833373	33.664939	44.871754	59.706482	80.388063
0.850	16.226683	23.157642	31.569765	42.230497	56.333544	75.988592
0.860	14.978914	21.616203	29.642989	39.801005	53.230027	71.939188
0.870	13.824713	20.194645	27.866754	37.560943	50.367610	68.203149
0.880	12.753577	18.880411	26.225509	35.490853	47.721616	64.748485
0.890	11.756171	17.662508	24.705656	33.573723	45.270462	61.547213
0.900	10.824100	16.531275	23.295262	31.794619	42.995204	58.574766
0.910	9.949701	15.478191	21.983819	30.140393	40.879155	55.809500
0.920	9.125827	14.495707	20.762043	28.599431	38.907571	53.232288
0.930	8.345607	13.577105	19.621707	27.161438	37.067387	50.826175
0.940	7.602125	12.716390	18.555496	25.817268	35.346986	48.576088
0.950	6.887935	11.908175	17.556889	24.558768	33.736018	46.468588
0.960	6.194178	11.147604	16.620055	23.378652	32.225230	44.491663
0.970	5.508668	10.430269	15.739767	22.270395	30.806331	42.634547
0.980	4.810711	9.752139	14.911325	21.228135	29.471878	40.887572
0.990	4.050571	9.109497	14.130494	20.246595	28.215166	39.242031
1.000	2.706025	8.498873	13.393443	19.321015	27.030150	37.690066

θ (the probability of observing a higher value at NOTL than at FE) and P_{00} (the probability that the two paired samples have values below the level of detection). As can be seen from Table 1, the value of M increases as θ decreases and as P_{00} increases. For example, to detect a change in θ from 0.5 to 0.55, 1077 and 1330 paired samples are required to be taken from the two stations when $P_{00} = 0$ and $P_{00} = 0.1$, respectively. It can also be noticed that weekly samples will allow the detection of change in θ from 0.5 to 0.715, while sampling once every 2 wk allows the detection of change from 0.5 to 0.79 in the value of θ. The changes in the probability can be transformed into changes in the centre of the distribution (median) in units of standard deviation if a particular form of probability distribution is assumed. This can be done by noting that

$$\theta = 1 - \Phi\left(-\frac{\Delta}{\sigma}\right)$$

where Φ is the cumulative distribution function and Δ and σ are the median and the standard deviation of the concentration differences. Hence

$$\Delta = -\sigma\Phi^{-1}(1 - \theta).$$

For example, if $\theta = 0.95$ and the distribution is normal, then

$$\Delta = 1.645\sigma.$$

Trend Detection and Estimation

Let y_{ij} and x_{ij} be the concentrations of substance A at NOTL and FE for the jth ($j = 1, 2, \ldots, n_i$) sample in the ith year ($i = 0, 1, 2, \ldots, k$) and suppose that r_i = the number of pairs in the ith year with $y_{ij} > x_{ij}$ and θ_i = the probability that $y_{ij} > x_{ij}$. Then the problem is testing

$$H_0\text{: } \theta_0 = \theta_1 = \ldots = \theta_k = \theta \text{ say.}$$

Let $\gamma_i = \theta_i - \theta, \quad i = 0, 1, \ldots, k$. Then

H_0: each $\gamma_i = 0$

H_1: at least one $\gamma_i \neq 0$

against

$$H_1\text{: } \theta_0 \neq \ldots \neq \theta_k.$$

This can be tested using the statistic

$$D^2 = \sum_{i=0}^{k} (r_i - n_i\hat{\theta})^2/n_i\hat{\theta}(1 - \hat{\theta})$$

where

$$\hat{\theta} = t_1/n_.$$

where $t_1 = r_0 + r_1 + \ldots + r_k$ and $n_. = n_0 + \ldots + n_k$. Under H_0 the distribution of D^2 is well approximated by the χ^2 distribution with k degrees of freedom.

This test is a general test for the homogeneity of the θ_i's. More specialized tests will perform better if different forms for H_1 are considered. The logistic transform (Cox 1970) is more likely to perform better for testing for trend. The linear logistic model is given by

$$\theta_i = e^{a+bi}/(1 + e^{a+bi}) \qquad (i = 0, \ldots, k)$$

where a and b represent the intercept and the slope of the linear logistic model. In this case, the interest is testing

$$H_0\text{: } b = 0$$

against

$$H_1\text{: } b \neq 0.$$

The test statistic, which has approximately a standard normal distribution, for doing this is derived in the appendix and is

$$Z = (t_2 - E(t_2|t_1))/\sqrt{V(t_2|t_1)}$$

where

$$t_2 = \sum_{i=0}^{k} ir_i$$

$$t_1 = n_.\hat{\theta}$$

$$E(t_2|t_1) = \hat{\theta} \sum_{i=0}^{k} in_i$$

$$V(t_2|t_1) = \frac{\hat{\theta}(1 - \hat{\theta})}{n_. - 1}\left(\sum_{i=0}^{k} i^2 n_i - \frac{\left(\sum_{i=0}^{k} in_i\right)^2}{n_.}\right).$$

Estimation of the Number of Years Needed for Detecting the Existence of a Linear Trend

In this section the estimation of the number of years k necessary for monitoring the head and the mouth of the river in order to detect a specific change in $p[y_{ij} < x_{ij}] = \theta_i$ is considered. The difference in these probabilities, on the logistic scale, between year 0 and year k (Cox 1970) is given by

$$\ln\{(\theta_k/(1 - \theta_k))/(\theta_0/(1 - \theta_0))\} = bk.$$

To use the procedure given previously, the conditional distributions of t_2 given t_1 under H_0 and H_1 are required. Although these distributions can be derived in general, the exact expressions for the moments under H_1 are, however, not known. To avoid this difficulty, the score statistics procedure (Harris 1985; Plackett 1974) is used. This is done assuming $n_i = n$ for each of $k + 1$ years and by noting that t_1 and t_2 have asymptotically a bivariate normal distribution with means

$$\mu_1 = E(t_1) = n \sum_{i=0}^{k} \frac{e^{a+bi}}{1 + e^{a+bi}} = n \sum_{i=0}^{k} \theta_i$$

$$\mu_2 = n \sum_{i=0}^{k} i\theta_i.$$

The elements of the variance–covariance matrix are

$$\text{Var } (t_1) = I_{11} = n \sum_{i=0}^{k} \theta_i(1 - \theta_i)$$

$$\text{Var } (t_2) = I_{22} = n \sum_{i=0}^{k} i^2\theta_i(1 - \theta_i)$$

$$\text{Cov } (t_1, t_2) = I_{12} = n \sum_{i=0}^{k} i\theta_i(1 - \theta_i).$$

The conditional mean and variance of t_2 given t_1 are, respectively

$$\mu_{2.1} = E(t_2|t_1) = \mu_2 + (I_{12}/I_{11})(t_1 - \mu_1)$$

$$I_{22.1} = \text{Var } (t_2|t_1) = I_{22} - I_{12}^2/I_{11}.$$

The score statistic is

$$Z = (t_2 - \tilde{\mu}_{2.1})/\sqrt{\tilde{I}_{22.1}}$$

which has asymptotically a normal distribution with mean 0 and variance 1. The use of a tilde over $\mu_{2.1}$ and $I_{22.1}$ indicates that these quantities are evaluated under H_0. In this case

$$H_0\text{: } b = 0$$

which implies that $\theta_i = \theta_0$ for all i. Hence

$$\tilde{\theta}_0 = t_1/n_.$$

where $n_. = n(k + 1)$. Similarly

$$\tilde{I}_{22.1} = \frac{n_.\tilde{\theta}_0(1 - \tilde{\theta}_0)k(k + 2)}{12}.$$

Note that if $n_. - 1$ is replaced by $n_.$ in the expression for Var (t_2), then $\tilde{I}_{22.1}$ is obtained. Approximations to the conditional mean and variance of t_2 can be obtained using a procedure due to Cox (1970). This procedure gives

$$E(t_2|t_1) \simeq \tilde{\mu}_{2.1} + b\tilde{I}_{22.1} + \frac{1}{2}b^2\tilde{\mu}_3(t_2|t_1) + \ldots$$

$$V(t_2|t_1) \simeq \tilde{I}_{22.1} + b\tilde{\mu}_3(t_2|t_1) + b^2[\tilde{\mu}_4(t_2|t_1) - 3\tilde{I}_{22.1}^2] + \ldots$$

where $\tilde{\mu}_3(t_2|t_1)$ and $\tilde{\mu}_4(t_2|t_1)$ are the third and fourth central conditional moments of t_2 under H_0. It is easy to verify that $\tilde{\mu}_3(t_2|t_1) = 0$. Hence, ignoring terms of order b^2 in the above expressions, the number of years k is estimated, assuming that

TABLE 2. Number of years required for detecting linear trend using the logistic model.

No. of years k	b	
	$n = 26$	$n = 52$
1	1.8196	1.2867
2	0.9096	0.6432
3	0.5752	0.4067
4	0.4068	0.2877
5	0.3075	0.2174
6	0.2431	0.1719
7	0.1985	0.1404
8	0.1661	0.1175
9	0.1416	0.1001
10	0.1227	0.0868
20	0.0464	0.0328
50	0.0122	0.0086

the rates of type I and type II errors are equal to 0.05, by solving the equation

$$b^2\tilde{I}_{22.1} = 4(1.645)^2$$

$$b^2 = 129.889/[(n\tilde{\theta}_0)(1 - \tilde{\theta}_0)k(k + 1)(k + 2)]$$

which is a cubic equation in k. It should be noted that the function $\tilde{\theta}_0(1 - \tilde{\theta}_0)$ is symmetric about $\tilde{\theta}_0 = 0.5$; hence, the value of k satisfying the above equation is the same for the two values $\tilde{\theta}_0 = 0.5 + \epsilon$ and $\tilde{\theta}_0 = 0.5 - \epsilon$. Furthermore, $\tilde{\theta}_0(1 - \tilde{\theta}_0)$ is nearly constant within the range $0.3 \leq \tilde{\theta}_0 \leq 0.7$. Hence, vague knowledge about $\tilde{\theta}_0$, when small deviation from H_0 is expected, will have a small effect on the estimated value of k.

It is simpler to calculate the value of b for each value of k. This is given in Table 2 when $\tilde{\theta}_0 = 0.5$ and for $n = 26$ and $n = 52$. It is clear that the number of years increases as b decreases and n increases. Monitoring for 3 yr will allow the detection of values of $b = 0.5752$ and 0.4067 when $n = 26$ and 52, respectively.

Discussion

This paper presents a set of novel statistical techniques for solving the important problem of estimating the frequency of sampling required for monitoring the Niagara River. Two aspects are considered. The first is to provide a technique for estimating the frequency of sampling the river at FE and NOTL within a given year, and the second is to estimate the number of years required for monitoring the river in order to detect a linear (on the logistic scale) trend in its water quality. The occurrence of data below the detection level especially for toxic contaminants made it appropriate to deal with this problem from a hypothesis testing point of view using nonparametric methods. This has resulted in the development of novel formulas for determining the sampling frequency.

References

COX, D. R. 1970. Analysis of binary data. Methuen, London. 142 p.

PLACKETT, R. L. 1974. The analysis of categorical data. Griffin, London. 159 p.

HARRIS, P. 1985. An asymptotic expansion for the null distribution of the efficient score statistic. Biometricka 72(3): 653–659.

Appendix

Under the logistic model the likelihood function for a and b is

$$L = {}_{n_0}C_{r_0} \cdots {}_{n_k}C_{r_k} \frac{e^{at_1 + bt_2}}{\prod_{i=0}^{k} (1 + e^{a+bi})^{n_i}}$$

where

$$t_1 = \sum_{i=0}^{k} r_i$$

$$t_2 = \sum_{i=0}^{k} ir_i.$$

The statistics t_1 and t_2 are jointly sufficient for a and b, respectively. Inference about b can be made using the conditional distribution of t_2 given t_1, or equivalently the conditional likelihood function for b which is

$$L_c = \frac{{}_{n_0}C_{r_0} \cdots {}_{n_k}C_{r_k} e^{bt_2}}{\sum_{r_0, \ldots, r_k} {}_{n_0}C_{r_0} \cdots {}_{n_k}C_{r_k} e^{bt_2}}$$

$$\Sigma\, r_i = t_1.$$

Under H_0, L_c becomes

$$L_c(H_0) = \frac{{}_{n_0}C_{r_0} \cdots {}_{n_k}C_{r_k}}{{}_{n_\cdot}C_{t_1}}.$$

The moments of t_2 can then be calculated from $L_c(H_0) = P(r_0, \ldots, r_u | t_1)$. It is easy to verify that the conditional mean and variance of t_2 are, respectively

$$E(t_2|t_2) = \sum_{i=0}^{k} \frac{in_i}{n_\cdot} t_1$$

$$= \hat{\theta} \sum_{i=0}^{k} in_i$$

$$V(t_2|t_1) = \frac{\hat{\theta}(1 - \hat{\theta})}{n_\cdot - 1}\left(n_\cdot \sum_{i=0}^{k} i^2 n_i - \left(\sum_{i=0}^{k} in_i\right)^2\right)$$

$$= \frac{n_\cdot \hat{\theta}(1 - \hat{\theta})}{n_\cdot - 1}\left(\sum_{i=0}^{k} i^2 n_i - \frac{\left(\sum_{i=0}^{k} in_i\right)^2}{n_\cdot}\right).$$

Then the test for linear trend on the logistic scale is

$$Z = (t_2 - E(t_2|t_1))/\sqrt{V(t_2|t_1)}$$

which has approximately normal distribution with mean 0 and variance 1. The statistic Z takes a simple form when $n_i = n$, since

$$E(t_2|t_1) = \hat{\theta} n \frac{k(k + 1)}{2}$$

$$V(t_2|t_1) = \frac{n_\cdot \hat{\theta}(1 - \hat{\theta})}{n_\cdot - 1} n\left(\sum_{i=0}^{k} i^2 - \frac{\left(\sum_{0}^{k} i\right)^2}{k + 1}\right)$$

$$= \frac{nn_\cdot \hat{\theta}(1 - \hat{\theta})}{n_\cdot - 1}\left\{\frac{k(k + 1)(2k + 1)}{6} - \frac{k^2(k + 1)}{4}\right\}$$

$$= \frac{n_\cdot^2 k(k + 2)}{12(n_\cdot - 1)} \hat{\theta}(1 - \hat{\theta}).$$

Inverse Correlation between Ovarian Development of Atlantic Salmon (*Salmo salar*) Smolts and Sea Age

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In order to understand the factors which determine sea age of Atlantic salmon (*Salmo salar*), ovarian development of smolts was examined in 14 groups which varied in parental sea age, smolt age, and size. Parental sea age of smolts explained most of the variation in ovarian development. Smolts from one-sea-winter parents had a higher percentage of the more advanced oocyte stages in their ovaries, while smolts from two-sea-winter parents had a low percentage, and those from three-sea-winter parents had none. Annual within-stock variation and covariance with freshwater age were not significant. Hatchery-reared smolts had similar ovarian development to their wild counterparts. There was also a significant, positive correlation between fork length of smolts and ovarian development within groups.

Afin de comprendre les facteurs qui déterminent l'âge marin du saumon de l'Atlantique (*Salmo salar*), on a étudié le développement des ovaires de saumoneaux appartenant à 14 groupes de divers âges, tailles et âges parentaux marins. L'âge parental marin des saumoneaux explique dans une large mesure la variation du développement des ovaires. Les saumoneaux issus de parents unibermarins montraient un pourcentage élevé d'oocytes de stade avancé dans leurs ovaires tandis que les saumoneaux issus de parent dibermarins en possédaient un plus faible pourcentage; par contre, les saumoneaux issus de parents tribermarins n'en possédaient aucun. La variation annuelle au sein d'un stock et la covariance avec l'âge en eau douce n'était pas significative. Le développement des ovaires était semblable chez les saumoneaux d'élevage et les saumoneaux sauvages. Il existait aussi une corrélation significative positive entre la longueur à la fourche des saumoneaux et le développement des ovaires à l'intérieur des groupes.

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Age at maturity of anadromous Atlantic salmon (*Salmo salar*) is highly variable: freshwater residence can range from 1 to 7 yr, and saltwater residence can be 1–4 yr before the first spawning. Most Atlantic salmon from Canadian rivers, however, spend 2–4 yr in freshwater and return to spawn after one winter at sea (one-sea-winter or 1SW salmon). Less than 40% of the mean 1980–84 Canadian harvest of 700 000 Atlantic salmon was large or multi-sea-winter (MSW) salmon. Because MSW salmon are more valuable than 1SW salmon, there is considerable interest in understanding the factors which determine the length of saltwater residence or sea age of Atlantic salmon (Meerburg 1986).

Recently, it was shown that ovarian development of Atlantic salmon smolts was greater in a stock where parents of the smolts were 1SW salmon than in several stocks where smolts were progeny of MSW salmon (Chadwick et al. 1986). These results were important because they suggested that sea age of Atlantic salmon was determined either by genetic factors (Naevdal 1983) or at least by those in freshwater, in contrast with other studies which supported the influence of factors in the marine environment (Saunders et al. 1983; Martin and Mitchell 1985).

In this study, we test the hypothesis that sea age of Atlantic salmon is inversely correlated with ovarian development of smolts. We expand the results of Chadwick et al. (1986): first, we examined a wider selection of stocks than in the previous investigation; second, annual and within-stock variation in ovarian development were determined by obtaining a second year of samples from three populations differing in parental sea age at first maturity; third, we examined the influence of smolt age and size on ovarian development; and fourth, to assess the relative importance of genetic and environmental factors in controlling ovarian development, progeny from the wild 1SW stock previously examined by Chadwick et al. (1986) were reared in a hatchery at an accelerated growth rate and then compared with the wild stock after a second year of sampling.

Materials and Methods

To assess the influence of sea age on ovarian development of Atlantic salmon smolts, we examined wild and hatchery-reared stocks located throughout Atlantic Canada (Fig. 1). Smolts were collected from the natal rivers of seven wild stocks; three of these stocks, Bartholomew River, South River, and Western Arm Brook, were sampled in 1984 and 1985 to study interannual variation in ovarian development; all others were sampled

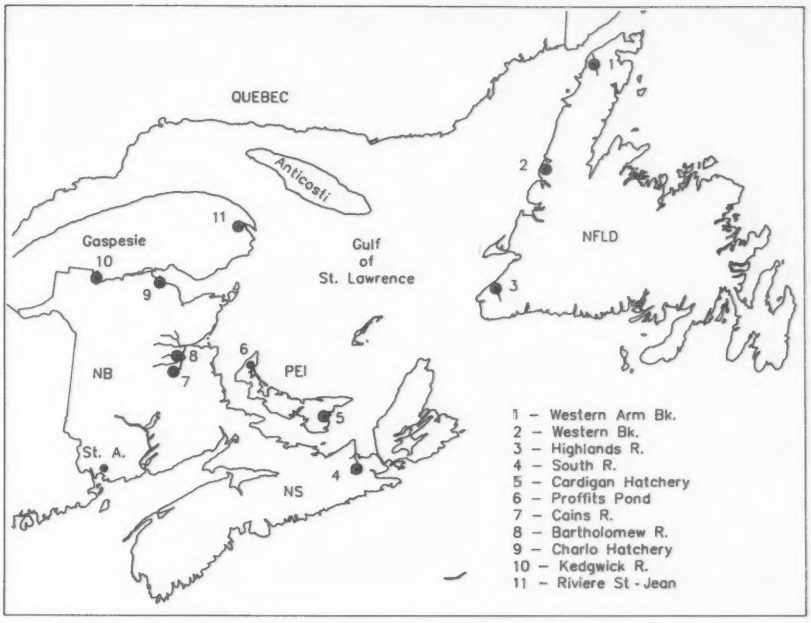

FIG. 1. Location of place names mentioned in text.

TABLE 1. Sea age of female parent, number sampled, sampling dates, and rearing conditions of the Atlantic salmon smolts collected from the 14 locations sampled during the study. Unless otherwise stated, smolts and adults were sampled in a similar manner.

Location	Sea age	*n*	Year	Sampling dates	Notes
Western Arm Brook (WAB), Nfld.	1.00	31	1984	June 11–14	Wild, sampled at counting fence
	1.00	91	1985	June 6 – July 13	Wild, sampled at counting fence
St. Andrews (STAND), N.B.	1.00	46	1985	April 29	Hatchery, progeny of Western Arm Brook stock
Western Brook (WEST), Nfld.	1.00	32	1985	June 26 – July 15	Wild, sampled at counting fence
Cains River (CAINS), N.B.	1.51	28	1984	May 24	Wild, smolts sampled with a trapnet, adults sampled from sport fishery
Bartholomew River (BARTH), N.B.	1.83	27	1984	May 22	Wild, sampled at counting fence
	1.84	48	1985	May 7 – 18	Wild, sampled at counting fence
Highlands River (HIGH), Nfld.	1.87	25	1984	June 4 – 9	Wild, adults sampled at counting fence, smolts sampled by angling
Cardigan Hatchery (CARD), P.E.I.	2.00	29	1985	April 25	Hatchery, progeny of northwest Miramichi stock
Profitt's Pond (PROF), P.E.I.	2.00	51	1985	April 24	Hatchery, reared at Cardigan Hatchery to 1+, seminatural pond rearing to 2+, progeny of northwest Miramichi stock, parental stock identical to Cardigan Hatchery
Rivière St-Jean (R.ST-JEAN), Que	2.10	31	1985	May 30	Wild, sampled at counting fence, sex ratio and fork length from counting fence and sport fishery 1981 only, age composition counting fence 1980–82
South River (SOUTH), N.B.	2.23	17	1984	May 11	Wild, sampled at counting fence
	2.23	43	1985	May 19	Wild, sampled at counting fence, adult migration sampled only in 1982
Charlo Hatchery (CHARLO), N.B.	3.00	11	1984	March 12	Hatchery, progeny of Kedgwick River stock

once (Table 1). Interstock variation in mean sea age of female parents ranged from 100% 1SW salmon to a stock which was 80% two-sea-winter (2SW) salmon and 20% three-sea-winter (3SW) salmon (Table 1). Mean sea age was estimated for the parents of wild smolts using samples collected by angling and at counting fences (Table 1). Sea age was determined from scales. Sex was determined from external characteristics on Rivière St-Jean, Western Brook, and South, Highlands, and Bartholomew rivers. On the other rivers, sex was determined surgically. The mean sea age of female parents was weighted by the percentage of eggs produced by each sea age class (Chadwick et al. 1986). For example, on Rivière St-Jean, 90% of the eggs were estimated to have been spawned by 2SW salmon and 10% by 3SW salmon. Hence, the mean sea age of

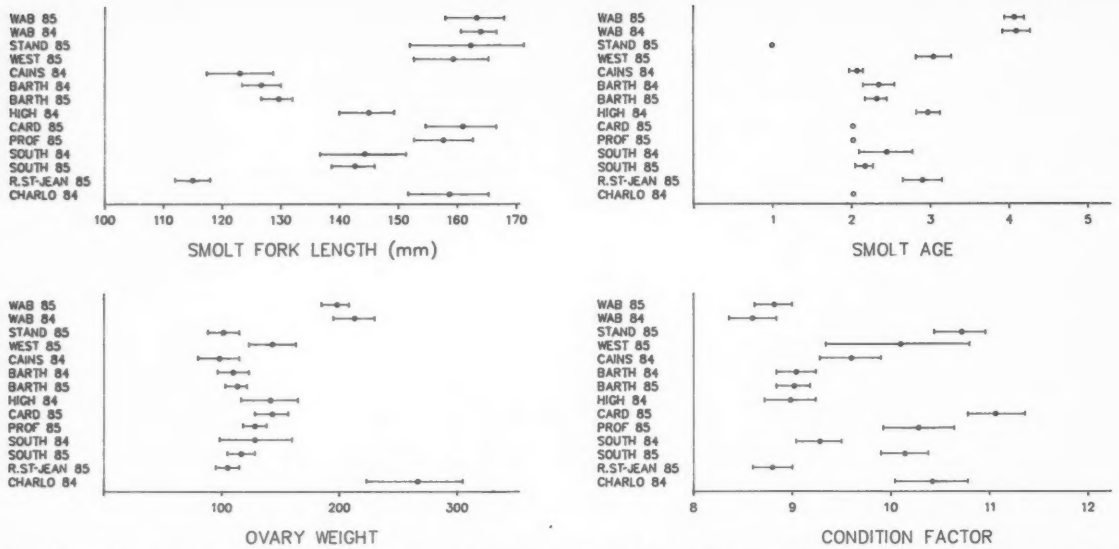

FIG. 2. Mean fork length, age, ovarian weight, and condition factor of 14 groups of smolts sampled in 1984 and 1985; 95% confidence limits are also indicated. See Table 1 for abbreviations.

female parents for these smolts was 2.10 yr. It was assumed that progeny of 1SW, 2SW, and 3SW salmon had equal survival rates. For South River, data were only available for the 1982 spawning migration, which accounted for 84% of the smolts in the 1985 sample. We assumed that this migration would be representative of the spawning migrations relevant to the 1984 smolt sample.

Smolts were also collected from three hatchery-reared stocks of 1SW, 2SW, and 3SW parents. Smolts from the 2SW stock were progeny of wild Western Arm Brook parents and were reared as age 1+ smolts at the Biological Station in St. Andrews, N.B. Wild Western Arm Brook smolts are, on average, 70% 4+ smolts (Chadwick 1981); they were compared with the accelerated smolts to see if a large (four-fold) reduction in smolt age would affect ovarian development. Smolts from the 2SW stock were progeny of wild Northwest Miramichi River parents and were reared as age 2+ smolts at Cardigan Fish Culture Station. At age 1+, these fish were separated into two groups: one group was raised in the hatchery and the other group was raised seminaturally, i.e. with a mixture of artificial and natural feeding in a small pond 200 km northeast of the hatchery (Profitts Pond). Smolts from the 3SW stock were progeny of wild Kedgwick River parents and were reared as age 2+ smolts at Charlo Fish Culture Station.

At the time of sampling, smolts were measured for fork length (±0.1 cm) and weight (±0.1 g) and a scale sample was removed for ageing. The left ovary was then excised, weighed (±0.01 g), fixed in Bouin's solution, and stored in 70% ethanol.

The histological procedure followed in 1984 is detailed in Chadwick et al. (1986). In 1985, the procedure was altered as follows: (1) the embedded ovaries were sectioned at 10 rather than 8 μm; (ii) the stained sections were studied using a high-resolution video camera and monitor system mated to a compound microscope, rather than directly through the microscope; (iii) a single complete section was studied and every nucleated oocyte was considered, rather than studying transects through several sections. Criteria used to define stage of oocyte development are outlined in Chadwick et al. (1986). None of the technical modifications is considered to have introduced variation in the identification of oocyte development stages in 1985 in comparison with those determined by the procedure used in 1984.

The null hypotheses that percentage of oocytes at each stage, mean stage of oocyte development, freshwater age, fork length, ovarian weight, and condition factor ($(W/FL^3) \times 100$) were equal among all populations were tested using analysis of variance (ANOVA) and Scheffé's procedure (Sokal and Rohlf 1981). Because stage 1 oocytes were few in number (<0.1%), they were combined with stage 2 counts. Stage 6 oocytes were observed in approximately one third of the ovaries examined and never represented more than 7% of the oocytes in any population. Hence, stage 6 oocytes were considered to be few in number and were combined with stage 5 oocytes prior to analysis. The proportions of stages 2 (1 + 2), 3, 4, and 5 (5 + 6) were arcsine transformed (Sokal and Rohlf 1981) before analysis to satisfy the normality and homogeneity of variance assumptions of ANOVA. Mean stage of oocyte development was weighted by the proportion of each stage in the population. ANOVA, Scheffé procedures, normality, and heterogeneity of variance tests were performed using SPSS/PC, Version 1.1 (Microsoft Corp., Chicago, IL 60611).

Regression analyses were used to examine correlations of ovarian development with fork length, sea age, and freshwater age. Regressions on $\log_e$-transformed fork length were calculated for individual groups and all groups combined. Regressions on sea age and freshwater age were calculated using mean values of ovarian development for individual groups.

Results

Fork length, ovarian weight, smolt age, and condition of smolts varied greatly among stocks (Fig. 2), but this variation could not be explained by differences in parental sea age. For

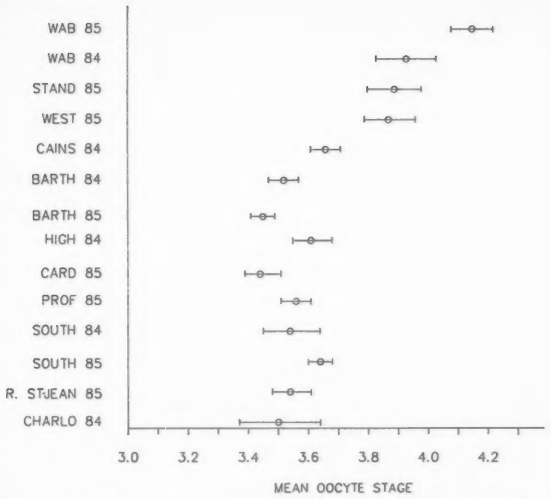

FIG. 3. Mean oocyte development stage (oocyte stage) and 95% confidence limits for 14 groups of smolts sampled in 1984 and 1985. STAND 85 refers to smolts of wild Western Arm Brook 1SW salmon parents which were reared in St. Andrews Biological Station and sampled as 1+ smolts in 1985. CHARLO 84 refers to smolts of wild Kedgwick River 3SW salmon parents which were reared at Charlo Fish Culture Station and sampled as 2+ smolts in 1984. Other abbreviations are given in Table 1.

example, mean fork length of smolts from the two 1SW stocks and the three hatchery-reared stocks (1SW, 2SW, and 3SW) was not different; and ovarian weight was significantly greater ($P < 0.05$) in Charlo (3SW) and Western Arm Brook (1SW) than all other stocks. It was also noteworthy that fork length was not different between the oldest (age 4+) and youngest (age 1+) smolts.

Variation in ovarian development among stocks, however, was better explained by differences in parental sea age. Ovaries were more developed from stocks in which the parents were 1SW salmon than those from stocks with 2SW and 3SW parents. The mean ovarian development stage for smolts from the 1SW stocks (Western Arm Brook, 1984, 1985; Western Brook, 1985; St. Andrews, 1985) was greater ($P < 0.001$) than all other groups (Fig. 3). Generally, the 1SW stocks had more stage 5 and 6 oocytes and fewer oocytes at stage 4 than the other stocks (Fig. 4). All 2SW and 3SW stocks had <10% stage 5 and 6 oocytes in their ovaries. Hatchery-reared smolts from the one 3SW stock had no stage 5 and 6 oocytes in their ovaries. There were no significant differences between stocks for stages 2 and 3. Mean oocyte development stage and the proportion of stage 5 oocytes were inversely correlated with parental sea age (Fig. 5 and Table 2, respectively), and the proportions of stage 2, 3, and 4 oocytes were positively correlated with sea age (Table 2).

Freshwater environment was less related to ovarian development than parental sea age. Two examples clearly make this point. First, ovarian development was not different between the groups of smolts with the fastest and slowest freshwater growth rates; both groups were offspring of 1SW parents. The mean oocyte development stage and the proportion of oocytes at stages 2, 3, and 5 were not different between age 1+ Western Arm Brook smolts raised at the Biological Station in St. Andrews and their wild age 4+ counterparts (Fig. 3 and 4).

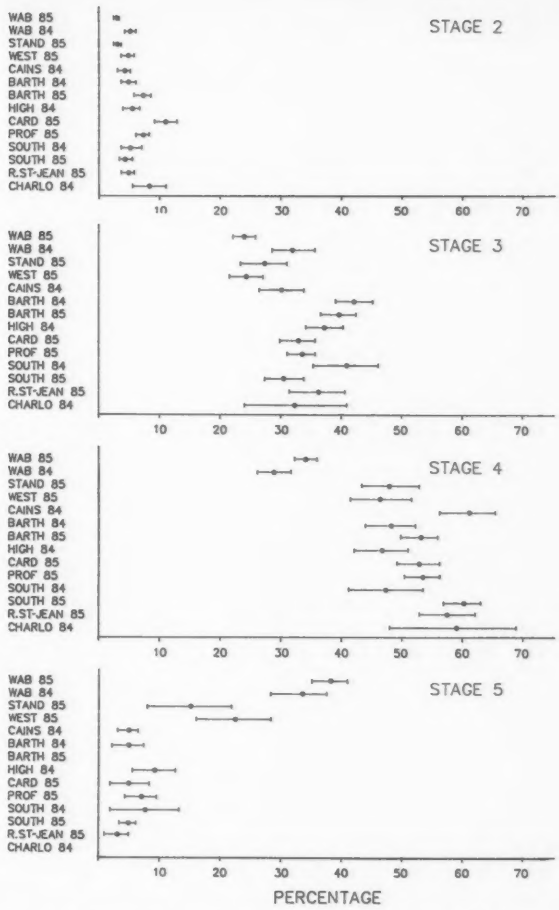

FIG. 4. Percentage of oocytes at stages 1 or 2, 3, 4, and 5 or 6 for 14 groups of smolts sampled in 1984 and 1985. See caption to Fig. 3 for further details.

The proportion of oocytes at stage 4 was not different between the hatchery-reared Western Arm Brook smolts and the other 1SW stock, Western Brook (Fig. 4). Second, Western Brook (1SW), Highlands River (2SW), and Rivière St-Jean (2SW) all had predominantly age 3+ smolts (Fig. 2), but the ovarian development of Western Brook smolts was significantly greater (Fig. 3 and 4) than the two stocks with 2SW parents.

Annual variation in oocyte development was not consistent. The mean ovarian development stage was different between 1984 and 1985 for smolts from Western Arm Brook but not for Bartholomew and South rivers (Fig. 3). In more detail, the percentage of stage 2, 3, and 4 oocytes was significantly different between 1984 and 1985 for Western Arm Brook smolts (Fig. 4). On South River, the percentage of stage 3 and 4 oocytes was different between smolts sampled in 1984 and 1985. Finally, on Bartholomew River, only stage 5 oocytes were different between the two years (Fig. 4).

There was an indication that some of the within-stock variation in oocyte development could be explained by fork length of smolts (Fig. 2). The largest smolts were those reared in hatcheries (Charlo, Cardigan, Profitt's Pond, and St. Andrews)

TABLE 2. Summary of regressions between mean oocyte development stage and fork le
in 1984–85. Slopes are given only for significant regressions.

	Mean stage			2		
Group	Slope	R^2	P	Slope	R^2	P
1. Fork length ($\log_e$)						
Overall	2.62	0.29	0.0000		0.01	0.0938
WAB 85	2.96	0.15	0.0002		0.00	0.5461
WAB 84	4.18	0.36	0.0004		0.01	0.6464
STAND 85	3.09	0.72	0.0000		0.00	0.7459
WEST 85	2.72	0.32	0.0012		0.00	0.7521
CAINS 84		0.00	0.9529		0.04	0.3275
BARTH 84		0.05	0.2528		0.01	0.7021
BARTH 85		0.05	0.1087		0.04	0.1843
HIGH 84		0.02	0.5466		0.11	0.1091
CARD 85		0.04	0.3240		0.12	0.0712
PROF 85	1.37	0.12	0.0155	−26.07	0.13	0.0102
SOUTH 84		0.02	0.5665		0.00	0.8843
SOUTH 85		0.07	0.0915	−65.60	0.29	0.0002
R. ST-JEAN		0.07	0.1636		0.00	0.9515
CHAR 84		0.21	0.1538		0.01	0.7269
2. Sea age						
Group mean	−0.28	0.65	0.0005	2.39	0.32	0.0342
3. Smolt age						
With STAND		0.28	0.0518		0.06	0.4105
Without STAND	0.25	0.72	0.0002	−1.89	0.31	0.0487

d fork length, parental sea age, and smolt age of 14 groups of smolts sampled in Atlantic Canada

	Stage of ovarian development								
	3			4			5		
P	Slope	R^2	P	Slope	R^2	P	Slope	R^2	P
938	−35.21	0.13	0.0000	−46.97	0.15	0.0000	106.39	0.29	0.0000
461	−29.86	0.05	0.0437	−36.01	0.08	0.0083	64.83	0.11	0.0016
464	−60.94	0.16	0.0264	−63.20	0.25	0.0042	126.63	0.39	0.0002
459	−66.90	0.46	0.0000	−51.63	0.24	0.0005	124.18	0.64	0.0000
521		0.03	0.3123	−74.82	0.22	0.0075	123.13	0.25	0.0039
275		0.06	0.2196		0.05	0.2463		0.00	0.7812
021		0.11	0.0866		0.02	0.4985		0.00	0.9364
843		0.02	0.3512		0.04	0.1526	—	—	—
091		0.01	0.5773		0.09	0.1398		0.09	0.1563
712		0.07	0.1687		0.00	0.9162	71.17	0.30	0.0441
102		0.04	0.1720		0.00	0.8350		0.11	0.0536
843		0.14	0.1409		0.12	0.1744		0.00	0.8367
002		0.03	0.2761		0.01	0.4648		0.03	0.3345
515		0.08	0.1261		0.07	0.1596		0.00	0.9058
269	168.81	0.38	0.0422		0.29	0.0875		—	—
342	3.11	0.31	0.0405	5.96	0.45	0.0084	−14.67	0.79	0.0001
105		0.02	0.6010	−4.69	0.52	0.0038		0.26	0.0931
487	−1.78	0.15	0.1990	−6.71	0.76	0.0001	11.97	0.77	0.0004

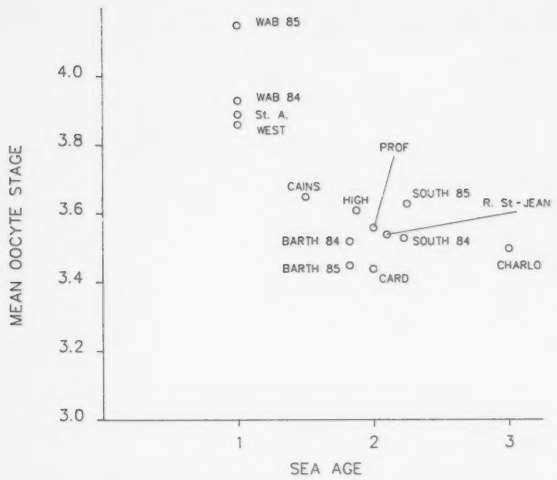

FIG. 5. Relationship between mean oocyte development stage (oocyte stage) and parental sea age for 14 groups of smolts sampled in 1984 and 1985.

and wild smolts from 1SW parents (Western Arm Brook and Western Brook). For the wild 1SW stocks, mean oocyte stage increased significantly with smolt size, the proportion of stage 3 and 4 oocytes declined with size, and the proportion of stage 5 oocytes increased with size (Table 2). Significant correlations of ovarian development with fork length were also found consistently at all stages in hatchery-reared smolts from St. Andrews and at some stages for smolts reared at Cardigan, Profitt's Pond, and Charlo (Table 2).

Discussion

We concluded that the hatchery environment did not influence sea age at first spawning because wild and hatchery-reared Western Arm Brook smolts had similar states of ovarian development. This finding conflicts with earlier studies which suggested that the faster growth of juvenile salmon, raised in hatcheries as offspring of 2SW parents, may lead to early maturation as grilse (Ritter et al. 1986). These other studies were based on the fact that a large proportion of tagged smolts were recovered as 1SW salmon, regardless of parentage. One possible explanation for this observation is that the exploitation rate of 2SW salmon relative to 1SW salmon was underestimated and few 2SW salmon survived to return to their natal rivers. The higher exploitation of 2SW salmon might also explain, in part, why most smolts released into sea cages became 2SW salmon, while smolts of the same parents released directly to the sea returned predominantly as 1SW salmon (Saunders et al. 1983). Saunders et al. (1983) had explained this observation with a model which required a negative correlation between winter sea temperature and sea age at maturity. An account of the relative roles of genetic and environmental factors in determining age at maturity of Atlantic salmon is given in Saunders (1986).

Another result of this study was that ovarian development did not covary with smolt age, but it did covary with fork length. Larger smolts in the wild 1SW stocks and all hatchery-reared stocks had ovaries which were more mature, i.e. more stage 5 and 6 oocytes, than smaller smolts within these stocks (Table 2). This result is corroborated by Ritter (1975) who found that larger hatchery-reared Atlantic salmon smolts were more likely to return as grilse, and Hager and Noble (1976) and Bilton et al. (1982) described a similar situation for smolts of *Oncorhynchus kisutch*. This result also provides a mechanism for a stock to change its sea age. For example, density-dependent growth might cause a crowded population to produce smolts which are smaller at a given age, and these smolts might be less mature and, consequently, spend longer at sea. A density-dependent shift in smolt size has already been described for Western Arm Brook (Chadwick 1987), but it is not yet known if this has had any influence on sea age. In conclusion, it appears that genetic factors, such as parental sea age, were more important in controlling ovarian development of smolts than environmental factors, such as growth rates in freshwater.

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References

BILTON, H. T., D. F. ALDERDICE, AND J. T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–447.

CHADWICK, E. M. P. 1981. Biological characteristics of Atlantic salmon smolts in Western Arm Brook, Newfoundland. Can. Tech. Rep. Fish. Aquat. Sci. 1024: 45 p.

1987. Causes of variable recruitment in a small Atlantic salmon stock. *In* M. J. Dodswell, R. J. Klauda, C. M. Moffitt, and R. L. Saunders [ed.] Common strategies of anadromous and catadromous fishes. Am. Fish. Soc. Symp. I. 570 p.

CHADWICK, E. M. P., R. G. RANDALL, AND C. LÉGER. 1986. Ovarian development of Atlantic salmon (*Salmo salar*) smolts and age at first maturity, p. 15–23. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89: 118 p.

HAGER, R. C., AND R. E. NOBLE. 1976. Relation of size at release of hatchery-reared coho salmon to age, size, and sex composition of returning adults. Prog. Fish Cult. 38: 144–147.

MARTIN, J. H. A., AND K. A. MITCHELL. 1985. Influence of sea temperature upon the numbers of grilse and multi-sea-winter Atlantic salmon (*Salmo salar*) caught in the vicinity of the River Dee (Aberdeenshire). Can. J. Fish. Aquat. Sci. 42: 1513–1521.

MEERBURG, D. J. [ED.] 1986. Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89: 118 p.

NAEVDAL, G. 1983. Genetic factors in connection with age at maturation. Aquaculture 33: 97–106.

RITTER, J. A. 1975. Relationships of smolt size and age with age at first maturity in Atlantic salmon. Environ. Can. Res. Dev. Branch Tech. Rep. Mar/T-75-5.

RITTER, J. A., G. J. FARMER, R. K. MISRA, T. R. GOFF, J. K. BAILEY, AND E. T. BAUM. 1986. Parental influences and smolt size and sex ratio effects on sea age at first maturity of Atlantic salmon (*Salmo salar*), p. 30–38. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89: 118 p.

SAUNDERS, R. L. 1986. The scientific and management implications of age and size at sexual maturity in Atlantic salmon (*Salmo salar*), p. 3–6. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89: 118 p.

SAUNDERS, R. L., E. B. HENDERSON, B. D. GLEBE, AND E. J. LOUDENSLAGER. 1983. Evidence of a major environmental component in determination of the grilse: larger salmon ratio in Atlantic salmon (*Salmo salar*). Aquaculture 33: 107–118.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry: the principles and practice of statistics in biological research. 2nd ed. W. H. Freeman and Co., San Francisco, CA. 859 p.

Patterns of Larval Emergence and Their Potential Impact on Stock Differentiation in Beach Spawning Capelin (*Mallotus villosus*)[1]

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The environmental cues triggering larval capelin (*Mallotus villosus*) emergence in the St. Lawrence estuary and in coastal Newfoundland are different. In the estuary, emergence from the intertidal and subtidal spawning grounds starts with dusk and ends with dawn, indicating an active response to low light intensity. In the laboratory, emergence from undisturbed sediments collected in the field is perfectly synchronized with the dark phase of the illumination cycle. Nocturnal emergence would represent an adaptation reducing vulnerability to visual predators. Previous work has shown that in Newfoundland, capelin emergence from intertidal beaches is triggered by abrupt changes in water temperature following the sporadic advection to the coast of surface water masses characterized by low predator and high prey abundances. We argue that regional differences in the emergence pattern of the species represent different strategies to avoid predation at the onset of the planktonic drift when the vulnerable yolk sac larvae are densely aggregated. Selection acting on the behaviour of the early larval stages could contribute to stock differentiation in capelin.

Les signaux environnementaux qui déclenchent l'émergence des larves de capelan (*Mallotus villosus*) dans l'estuaire du Saint-Laurent et sur la côte est de Terre-Neuve sont différents. Dans l'estuaire, les larves émergent des frayères littorales et sublittorales de la tombée du jour à l'aube, ce qui indique une réponse active aux faibles intensités lumineuses. En laboratoire, l'émergence est en synchronisme parfait avec la phase nocturne du cycle d'éclairement. Le caractère nocturne de l'émergence représenterait une adaptation visant à réduire la vulnérabilité des larves aux prédateurs visuels. Des travaux antérieurs ont montré qu'à Terre-Neuve, l'émergence du capelan des plages littorales est déclenchée par des variations brusques de la température de l'eau causées par l'advection sporadique à la côte de masses d'eau de surface riches en proies et pauvres en prédateurs. Nous soutenons que ces différences régionales dans le patron d'émergence de l'espèce représentent des stratégies différentes visant à réduire la prédation au début de la phase planctonique, alors que la densité des larves est très élevée. Une sélection agissant sur le comportement des stades larvaires initiaux pourrait contribuer à la différentiation des stocks chez le capelan.

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Several species of salmoniform, atheriniform, and tetraodontiform fish spawn in the intertidal zone where the eggs are periodically exposed to air. Spawning time, egg development, and the emergence of larvae of these species are often synchronized with the fortnightly cycle of tidal amplitude (Taylor 1984). In his review of lunar synchronization of fish reproduction, Taylor (1984) compiled the characteristics common to the reproductive strategy of most intertidal spawners. All species reviewed spawned during high spring tides, in intertidal areas that were accessible only during spring tide. The eggs could withstand exposure for several days. Hatching coincided with the following spring tide. In several species, the eggs hatched only when immersed, thus preventing premature emergence in air.

Capelin (*Mallotus villosus*) is a key forage species in the food web of the North Atlantic (Bailey et al. 1977; Akenhead et al. 1982; Carscadden 1983). Intertidal spawning is facultative in this species. Capelin of northern Europe, Iceland, and Greenland spawn in the sublittoral zone at depths of 10–70 m (Jangaard 1974). An exception is the isolated population of Balsfjord in northern Norway, which spawns inter-

[1]Contribution to the program of GIROQ (Groupe interuniversitaire de recherches océanographiques du Québec).

tidally (Davenport and Stene 1986). In the Northwest Atlantic, as opposed to northern Europe, capelin appears to spawn principally in the intertidal area. The adherent eggs are buried in beaches and intertidal flats along the coast of Newfoundland and the Gulf of St. Lawrence (Templeman 1948; Parent and Brunel 1976). Where intertidal spawning occurs, egg deposition is observed in the adjacent sublittoral zone as well (Templeman 1948; Winters 1969). Pitt (1958) reported evidence of deep spawning on the Grand Bank.

Egg development and the emergence of larvae from the beaches of eastern Newfoundland have been thoroughly studied by Frank and Leggett (1981a, 1981b, 1982a, 1982b, 1983, 1984). In this area, tidal amplitude is small (<1.5 m) and capelin spawn around spring tide. Hatching and the emergence of the larvae are not strongly linked to the following spring tide as in typical intertidal spawners. Time to hatching depends rather on incubation temperature. The eggs hatch in the gravel independent of tide condition and larvae may remain in the spawning substrate for several days. Active emergence into the aquatic environment coincides with abrupt temperature increases associated with the advection of warm surface waters by onshore winds (Frank and Leggett 1981a, 1983). The physiological condition of the larvae at the onset of the planktonic drift is negatively correlated with the time spent in the gravel (Frank and Leggett 1982a). The significant correlation between the frequency of onshore winds in June and year class strength supports the hypothesis that early survival and subsequent recruitment are adversely affected by a prolonged sojourn of the larvae in the spawning substrate (Leggett et al. 1984).

Capelin spawning is observed in almost every suitable locality along the coast of the estuary and Gulf of St. Lawrence (Parent and Brunel 1976). Intertidal spawning is particularly intense in the upper estuary (Parent and Brunel 1976), where tidal amplitudes average 4.5 m. Given these amplitudes, we hypothesized that larval capelin emergence in this region was closely synchronized with tidal signals, as in other intertidal spawners. To test this hypothesis, we monitored the embryonic development and the emergence of capelin larvae from the intertidal flats of the upper St. Lawrence estuary.

Materials and Methods

Area Studied

Capelin spawn on intertidal flats and beaches on both shores of the St. Lawrence estuary (Fig. 1a) (Parent and Brunel 1976). The preferred spawning substrate consists of coarse sand found at the confluence of small influents to the estuary (Parent and Brunel 1976). Deep spawning in the estuary has not been reported yet, but Parent and Brunel (1976) considered its occurrence to be possible if not probable.

The study was conducted in Saint-Irénée Bay, the most upstream location on the north shore where spawning is known to occur every year. In some years, spawning takes place at Ile aux Coudres, upstream of Saint-Irénée (Parent and Brunel 1976). Saint-Irénée Bay is well protected by hills and cliffs on the western and northwestern sides (Fig. 1b). Sand flats suitable for capelin spawning are located at the confluence of the Jean-Noël River which flows into the bay. There, the intertidal zone extends for approximately 500 m with a light slope of approximately 1%. The sand flats stretch out from the upper quarter of the intertidal zone to approximately 50 m beyond the

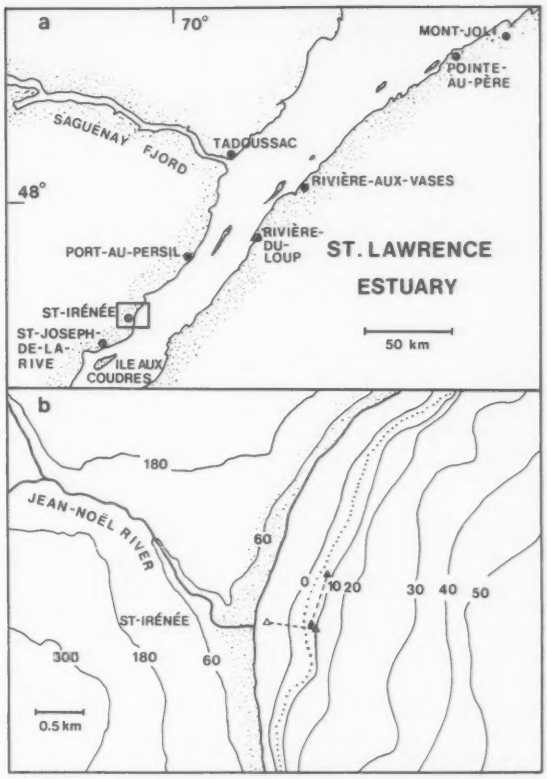

FIG. 1. (a) St. Lawrence estuary and (b) topographic map of the area sampled in 1980 and 1981. Heights of topographic contour lines are in metres above Highest High Water (large tides). Depths of isobaths are in metres below Lowest Normal Tide (3.13 m). Dotted isobath corresponds to Lowest Low Water line. Broken lines between symbols indicate sampling transects in 1980 (open symbols) and 1981 (solid symbols).

Lowest Normal Tide (LNT) line (isobath 0). Beyond that point, the slope increases rapidly (Fig. 1b).

Field Sampling

Egg deposition and development were monitored from May 20 to June 6, 1980. Egg samples were collected daily from six adjacent stations distributed at equidistant levels on the steepest side of the main spawning bank. Adjacent stations were separated by a vertical height of about 12 cm, and the rising tide reached the highest station about 50 min after reaching the lowest station. The six stations were representative of the vertical range within which the bulk of intertidal spawning occurred. Egg samples were scooped from the sediment at low tide. From each sample, 50 eggs were examined and classified according to five developmental stages. These stages corresponded to Fridgeirsson's (1976) subdivisions of capelin embryonic period except that our stage I comprised his stages I and II (Table 1). A weighted average developmental stage index ($\bar{s}$) was computed as a central measure of egg development for a given sample, (Frank and Leggett 1981b):

$$\bar{s} = \Sigma\, s_i n_i / \Sigma\, n_i \quad \text{for } i = 1\text{–}5$$

TABLE 1. Developmental stages used in the classification of capelin eggs. Time from fertilization to end of stage is for eggs incubated at a constant temperature of 7.2°C (Fridgeirsson 1976).

Stage	Characteristics	Time from fertilization to end of stage	Fridgeirsson's stage
I	Fertilization to formation of the blastula	1.6 d (39 h)	I + II
II	Gastrulation	3.5 d (84 h)	III
III	Organogenesis I; formation of head, eyes, first somites, midgut	6 d (144 h)	IV
IV	Organogenesis II; formation of heart, pigmentation of eyes	11 d (264 h)	V
V	Prehatching; head separates from yolksac, pigmentation of gut	21–22 d (504–528 h)	VI

where s_i is the ith developmental stage index (1–5) and n_i is the number of eggs classified in this developmental stage.

Sediments from the shallow area beyond the Lowest Low Water (LLW) mark were collected by hand at low tide during spring tide (May 30 to June 4) and cursorily examined for the presence of eggs.

The emergence of capelin larvae was monitored by surveying their abundance in the nearshore area above and off the spawning grounds. In 1980, larval capelin density was estimated at intervals of 3 h from 08:00 June 1 to 11:00 June 9, yielding a 195-h time series of 66 observations. Each sample consisted of two consecutive near-surface collections (1 m depth) using a 50-cm-diameter, 80-μm-mesh standard net equipped with a TSK flowmeter. The net was towed at 1.5 m/s (3 kn (5.6 km/h)) towards shore from a buoy anchored 300 m off the LNT line. The net was thoroughly rinsed and the sample was immediately preserved in 4% buffered formalin. A duplicate tow was made from the shore towards the anchored buoy, using the same technique. At low tide, depth along the transect varied from 10 m at the buoy to 2 m nearshore. The counts in the duplicate tows usually differed by less than 20% and were averaged. The efficiency of the standard net in capturing recently hatched capelin larvae has been studied by Fortier and Leggett (1983) who found no evidence of avoidance and concluded that estimates of larval capelin abundance and size distribution from the standard net compared well with those obtained with a bongo net.

In 1981, larval capelin abundance was monitored at 3-h intervals for 120 h from 12:00 May 23 to 09:00 May 28. A near-surface (1 m depth) sample and a near-bottom (within 1 m from the bottom) sample were taken in succession. To maintain a constant depth for the near-bottom sample, the net was towed along a transect parallel rather than perpendicular to the shore. The transect was marked with two buoys anchored 200 m off the LNT line. Depth along the transect varied from 4 to 9 m depending on the tide.

In both years, current speed, salinity, and temperature were recorded at 5-min intervals with an Aanderaa current meter located 20 m off the main spawning flat. The probes of the instrument were positioned at the approximate level of maximum egg deposition. In 1980, a thermistor chain (Aanderaa Instruments) was laid on the main spawning bank, with one thermistor located at each of the six levels where egg samples were taken. The recording interval was 5 min. Water height was recorded in Saint-Joseph-de-la-Rive (Fig. 1a). Wind and irradiance data were recorded in Mont-Joli (Fig. 1a).

All capelin larvae were sorted and enumerated. Thirty larvae taken at random from each sample were measured to the nearest 0.05 mm using a photographic technique (Zwanenburg 1982). Measurements of yolk sac size being imprecise with the photographic technique, the main axis of the yolk sac of 100 other larvae was measured to the nearest 0.1 mm under the dissecting microscope.

Isolation Experiment

On June 12, 1986, sediments containing capelin eggs were collected with as little perturbation as possible from the low intertidal zone of the spawning beach of Pointe-au-Père in the lower estuary of the St. Lawrence (Fig. 1a). Six centimetres of sediment was carefully placed into two rectangular plastic containers (78 × 58 cm × 23 cm deep) and incubated in a closed-circuit rearing apparatus. The sediments were permanently covered with 15 cm of seawater oxygenated with air diffusors and recirculated through a cooling system and a bactericide UV chamber. Fluorescent lights (daylight type) were automatically turned on from 04:30 to 20:45, thus reproducing the illumination cycle prevailing in July at these latitudes. Incident light intensity at the surface of the covering water was 6.14×10^{-4} $\mu E \cdot cm^{-2} \cdot s^{-1}$, a value that corresponded to approximately 0.18% of full sunlight in summer.

The emerging capelin were easily collected from the overlaying seawater with dip nets. The larvae displayed no avoidance reaction towards the slow-moving dip nets. Regular monitoring of the emergence began at 23:30 June 27 and ended at 08:30 July 6. Collections of larvae and measurements of water temperature were made hourly from 20:30 to 05:30 and at intervals of 3 h from 05:30 to 20:30. After 4 d of monitoring, bacterial growth developed in the sediment of one container which was discarded to avoid contamination. Only the results from the remaining container will be presented.

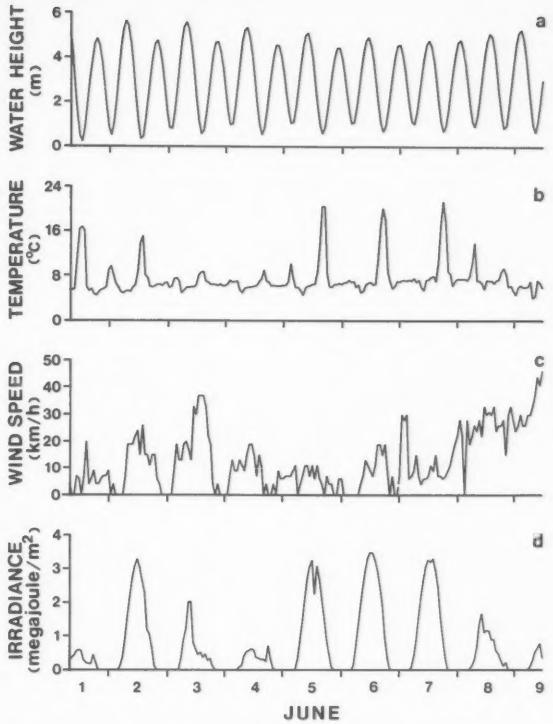

FIG. 2. Time series of environmental variables during the sampling period in 1980. (a) Water levels in Saint-Joseph-de-la-Rive, (b) temperature on the intertidal spawning beds in Saint-Irénée, (c) wind speed, and (d) solar radiation recorded in Mont-Joli. See Fig. 1 for locations.

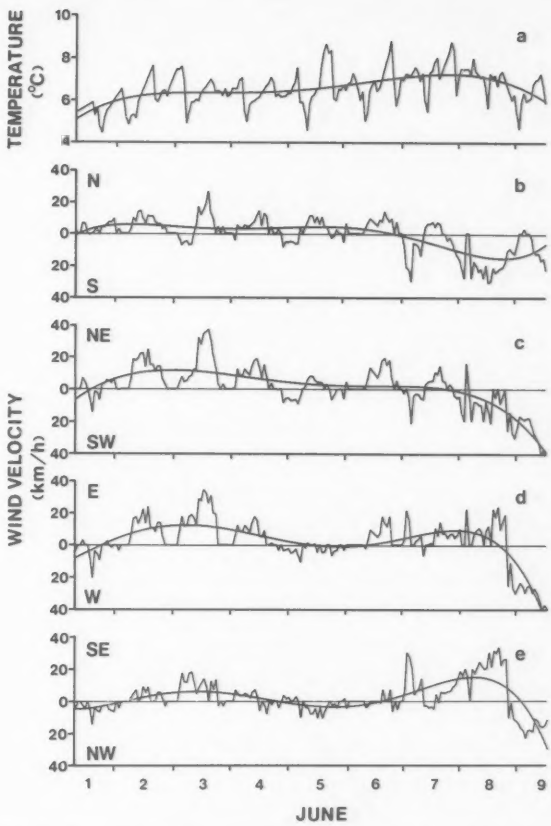

FIG. 3. Time series of (a) water temperature and (b–e) wind velocity from different azimuths during the sampling period in 1980. Trends were determined by fitting a polynomial function of time to the time series.

Results

Environmental Conditions in the Spawning Area

Semidiurnal tides were strong with a perceptible inequality in the amplitude of consecutive tides. Over the period monitored in 1980, tidal amplitude varied from 3.5 to 5.4 m at Saint-Irénée (Fig. 2a). Exposure of the spawning beds lasted for approximately 2.3 h on the small tide and 4.8 h on the large tide. At low tide on sunny days, temperature at the surface of the exposed beds reached 20°C (Fig. 2b). The temperature of the water covering the spawning beds averaged 6.5°C. Salinity of the water varied from 18 to 24‰, with maximum salinity recorded at high tide on the large-amplitude tides. Daylength was 16 h during the sampling period. Global solar radiation varied substantially from day to day with cloudiness (Fig. 2d).

Wind speed recorded in Mont-Joli presented a circadian cycle with maxima occurring around noon (Fig. 2c). This circadian cycle was also evident at the study site. Moderate northeasterly and easterly winds dominated from June 2 to June 4, followed by light and variable winds (Fig. 3c and 3d). During the last 2 d of monitoring, moderate southerly and southeasterly winds dominated (Fig. 3b and 3e).

In Saint-Irénée Bay, variations in water temperature were dominated by semidiurnal fluctuations but also contained a low-frequency trend (Fig. 3a). When the latter was removed, the residual fluctuations in water temperature were significantly correlated with water height ($r = -0.544$, $P < 0.001$). The low-frequency trend was positively correlated with the trend in the velocity of southeasterly and south-southeasterly winds ($r = 0.590$ and $r = 0.765$, respectively, $P < 0.001$), suggesting that low-frequency increases in water temperature resulted from the advection by onshore winds of warmer surface waters (Fig. 3).

Spawning and Egg Development

Egg deposition was not confined to the upper reaches of the intertidal zone as in typical intertidal spawners. Rather, the eggs were distributed in the lower half of the intertidal area on sand banks that were covered and exposed semidiurnally during the entire neap–spring tidal cycle. Sand from beyond the LLW mark contained egg concentrations that compared with those found in the intertidal zone. No eggs were found in the rocky or muddy zones that constitute the major part of the littoral.

Egg development followed a similar pattern at the six levels sampled in the intertidal zone (Table 2). The data were pooled to yield a general picture of embryonic development on the tidal flats (Fig. 4). Dominance of stage III and IV eggs on the first day of sampling (May 20) indicated that a spawning wave

TABLE 2. Weighted average developmental stage index ($\bar{s}$) of capelin eggs sampled at six adjacent levels on the main intertidal spawning bank. Station 1 corresponded to the highest level.

Date	Station					
	1	2	3	4	5	6
May 20	3.327	3.140	3.440	3.480	3.520	3.385
21	1.348	1.260	1.447	1.411	1.960	1.800
22	2.190	2.196	2.097	1.751	1.640	2.100
23	2.396	2.440	2.512	2.035	2.272	2.780
24	2.744	3.357	3.316	2.700	2.280	2.700
25	3.125	2.782	3.400	2.740	2.891	2.600
26	3.650	3.309	1.957	1.660	2.099	1.586
27	4.220	3.320	3.408	3.202	2.668	2.100
28	3.369	4.104	4.025	2.860	2.983	2.980
29	3.300	2.981	3.750	3.633	3.075	2.420
30	3.149	3.535	3.692	4.652	2.673	2.855
31	4.191	4.694	3.848	3.848	3.258	3.024
June 1	4.001	3.860	4.680	4.275	3.460	3.120
2	4.265	4.320	4.632	4.306	4.632	4.360
3	3.760	3.720	3.999	3.800	3.203	4.063
4	4.260	4.306	4.200	4.314	4.094	4.020
5	4.260	4.200	4.596	4.240	4.212	4.317
6	4.560	4.560	4.714	4.584	4.400	4.420

had taken place several days before sampling began. As 49% of the eggs were stage III and 42% were stage IV (Fig. 4), the egg population was just completing the transition between the two developmental stages. Frank and Leggett (1981b) have shown that the rate of development of capelin eggs incubating in the intertidal zone is governed by average incubation temperature. The relationship between hatching time and average incubation temperature established for eggs developing in situ was statistically indistinguishable from that for eggs incubated at a constant temperature in the laboratory (Frank and Leggett 1981b). This relationship is

$$\ln y = 4.27 - 0.63 \ln (x + 1)$$

where y = days to medium hatching and x = average incubation temperature. The temperature experienced by the eggs during the period of sampling averaged 7.8°C (mean of 25 926 measurements recorded at the six levels sampled from 16:00 May 20 to 16:00 June 4; SD = 3.55°C). Replacing this value in the above equation yields an estimate of 18.2 d for time to hatching. Assuming that time to the end of stage III is a constant proportion of time to hatching at any temperature (e.g. 6 d/21.5 d = 0.279, Table 1), the egg population collected on May 20 would have been about 5 d old (18.2 d × 0.279). Therefore, this first spawning wave probably occurred during the night from May 15 to May 16. The frequency histogram of stage I eggs indicated a second spawning during the night from May 20 to May 21. A third spawning wave in which egg deposition was restricted to the four lower levels sampled (Table 2) took place in the night from May 25 to May 26 during neap tide (Fig. 4).

Because the histograms represent frequencies rather than actual numbers at each stage, the maturation of these different cohorts of eggs is difficult to follow on Fig. 4. For example, the drop in the frequency of stages III and IV on May 21 resulted from an increase in the frequency of stage I eggs and did not necessarily indicate a decrease in the actual abundance of stages III and IV. The three distinct modes on the frequency histogram for stage V lagged the three spawning waves by 14, 13, and 12 d, respectively. If these modes reflected the progression of the three cohorts, the interval between fertilization and the middle of stage V varied from 12 to 14 d. These values are consistent with the period of 13.7 d predicted by Frank and Leggett's (1981b) equation, assuming that time from fertilization to the middle of stage V represents 75.6% of total development time (Table 1).

Emergence of the Larvae in the Field

In 1980, the regular monitoring of larval capelin abundance in the water column began on June 1. Several lines of evidence indicated that emergence began during the few days before that date: by May 25, a significant fraction of the eggs (~20%) had entered stage V (Fig. 4). On May 28, a few larvae were found in the sediment samples. By June 4, eggs became scarce on the intertidal flats while they remained abundant in the subtidal area.

Fluctuations in the abundance of larval capelin in the surface waters of the nearshore area were dominated by a circadian cycle. Abundance peaked at night, reaching concentrations of 400 larvae/m^3 (Fig. 5a). Relatively few larvae (<10/m^3) were caught in daytime except on a few occasions when up to 50 larvae/m^3 were captured. The day–night difference in average abundance was highly significant (Table 3).

The swarms of larval capelin observed near the surface at night were composed mostly of newly hatched yolk sac larvae characterized by relatively short lengths and large yolk sacs (Table 3; Fig. 5b and 5c). The frequency distributions of length and yolk sac size of larvae captured at night were significantly skewed towards shorter length and larger yolk sac when compared with those of larvae collected in daytime (Kolmogorov–Smirnov two samples test, $P < 0.01$) (Fig. 6). These characteristics indicated that the larvae captured at night were recently hatched and newly emerged from the sand flats. The few larvae captured in daytime had partially exhausted their yolk reserves and had slightly increased in length, suggesting an earlier emergence.

High densities of capelin larvae near the surface at night may be the result of vertical migration rather than nocturnal emergence. In 1981, samples were taken near the surface and

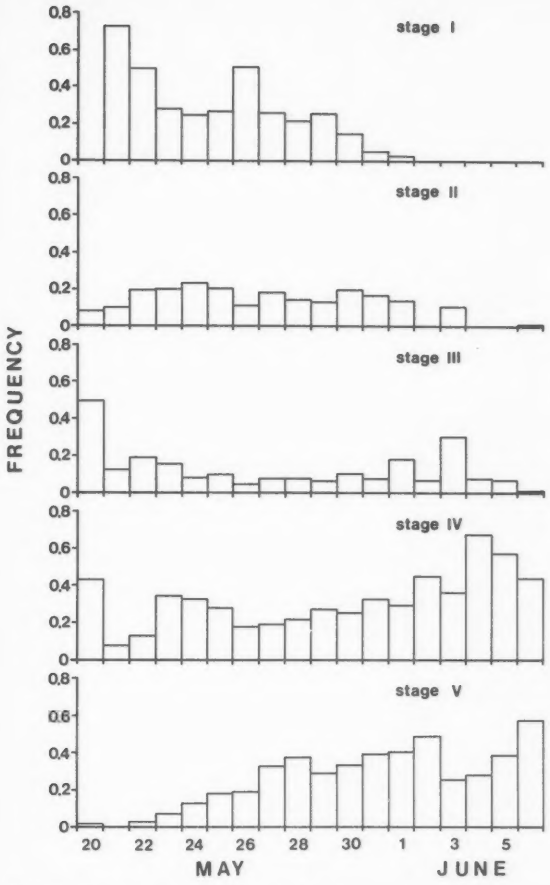

FIG. 4. Capelin egg development during the days preceding the sampling period in 1980. Histograms represent the frequency of a given developmental stage at a given date. Refer to Table 1 for stage descriptions.

near the bottom to verify the possible occurrence of vertical migrations bringing the larvae at depth in daytime, and therefore explaining the circadian cycle in near-surface abundance. The abundances recorded in 1981 were much lower than in 1980, indicating that sampling did not coincide with peak emergence in that year (Fig. 7). As in 1980, nighttime surface collections yielded higher abundances (t-test, $P < 0.01$) of larvae characterized by larger yolk sac (t-test, $P < 0.01$) than daytime surface collections. Few larvae were captured at either depths in daytime (Fig. 7), Thus, we found no evidence that emerging larvae concentrated near the bottom during the day. The low abundances in the nearshore water column in daytime suggested that larvae emerging at night were rapidly transported away from the spawning grounds.

In addition to the dominant circadian cycle, a low-frequency trend was visible in the 1980 time series of larval capelin abundance (Fig. 5a). Abundance was particularly high on the second and third nights of sampling. Nighttime abundance remained relatively low afterward, until a second increase on the sixth and seventh nights of sampling. The first wave of emergence (June 2 and 3) lagged the first spawning wave

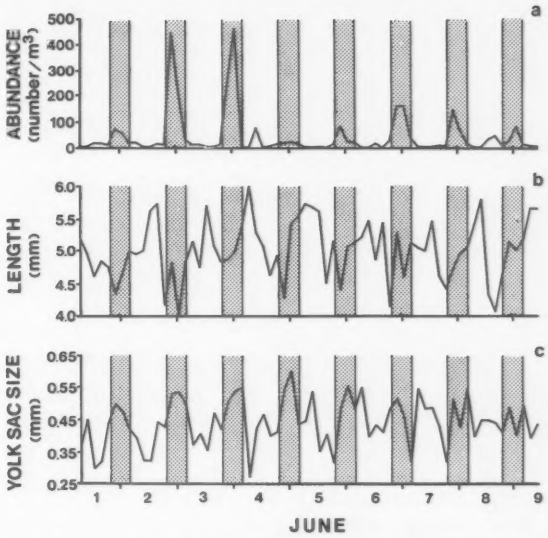

FIG. 5. Time series of larval capelin (a) abundance, (b) average length, and (c) average yolk sac size in the nearshore zone adjacent to the spawning beds in 1980. Shaded areas indicate nighttime.

(estimated date: night from May 15 to May 16) by 18–19 d. The second wave of emergence (June 6 and 7) followed the second spawning wave (night from May 20 to May 21) by 17–18 d. These intervals compare well with time from fertilization to hatching (18.2 d) predicted by Frank and Leggett's (1981b) equation for capelin eggs incubated at an average temperature of 7.8°C.

The samples were examined for the presence of larval fish predators. Few large invertebrates were present in the nearshore waters. In 1980, the overall 122 collections contained no gelatinous zooplankton, no euphausids, 3 chaetognaths, and 10 amphipods. In both years, adult capelin were regularly observed near the surface during sampling and frequently captured in the plankton nets. In 1981, 60 adults were caught in this manner, most of them spent (and probably weakened) males (66%). Gut content examination revealed that eight of these adults (13%) had preyed on larval capelin. The number of larvae in guts containing larvae varied from 2 to 15, with the exception of one spent male that ingested 335 larvae. Ninety-three percent of the larvae were found in the gut of 5 of the 14 adults captured during the night from May 24 to 25, coincident with the only important peak of emergence for the period monitored in 1981 (Fig. 7). Capelin larvae were found in the gut of spent males (13%), mature males (17%), and mature females (17%). No larvae were found in the gut of the four spent females captured. Adult capelin captured in 1980 were not preserved and, hence, were not available for gut content analysis.

Environmental Triggering of Capelin Emergence

The relationships between the emergence of capelin larvae and environmental factors were studied by relating the abundance of larvae in the nearshore zone to meteorological and hydrological variables. First, the heterogeneity of variance in the time series of capelin abundance was reduced by log-

TABLE 3. Day–night comparison of the ecophysiological characteristics of capelin larvae captured. $\bar{X}$ = mean; s^2 = variance; *Behrens–Fisher t.

		Day	n	Night	n	t	F	P
Abundance (number/100 m^3)	$\bar{X}$	2.864	42	3.750	24	6.39	—	<0.01
(log-transformed)	s^2	0.261	42	0.352	24	—	1.35	>0.10
Length (mm)	$\bar{X}$	5.078	1260	4.911	720	5.62*	—	<0.01
	s^2	0.495	1260	0.351	720	—	1.41	<0.01
Yolk sac size (mm)	$\bar{X}$	0.436	3331	0.480	2479	10.46*	—	<0.01
	s^2	0.029	3331	0.020	2479	—	1.45	<0.01
Yolk sac frequency	$\bar{X}$	0.712	42	0.840	24	2.928	—	<0.01
	s^2	0.029	42	0.032	24	—	1.10	>0.25

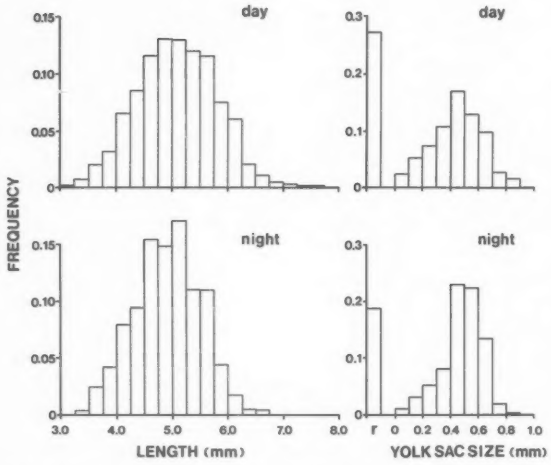

FIG. 6. Capelin length and yolk sac size frequency distributions for day and night collections. "r" stands for complete resorption. Means and variances are given in Table 3.

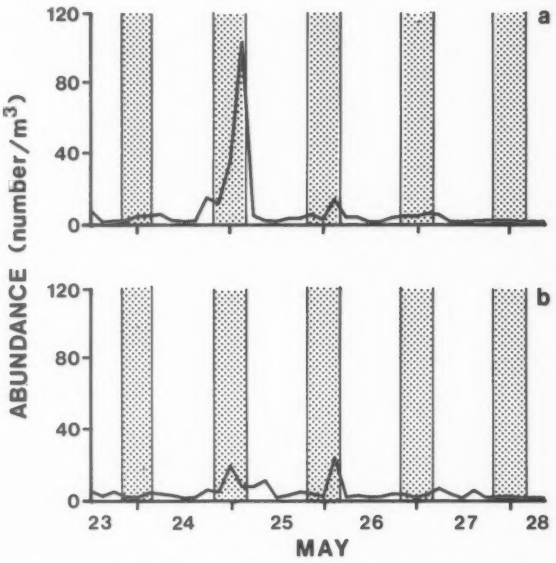

FIG. 7. Time series of larval capelin abundance in (a) near-surface and (b) near-bottom collections in 1981. Shaded areas indicate nighttime.

arithmic transformation (Fig. 8a). The low-frequency bimodal trend was isolated by fitting a polynomial function of time to the time series of log-normalized abundance (Fig. 8a). This trend was then subtracted from the raw log-normalized abundance data to obtain a stationary time series of capelin abundance that comprised only the dominant circadian rhythm and the higher frequency variations (Fig. 8b).

The stationary variations in capelin abundance were strongly and negatively correlated with light intensity ($r_{max} = -0.723$, $P < 0.01$). The evening increase and the morning decrease in larval capelin abundance coincided with dusk and dawn, respectively (Fig. 8b).

No significant link was found betwen the stationary time series of larval capelin abundance and the time series of hydrological variables characterized by a semidiurnal period such as water height, salinity, or current speed ($r \leq 0.091$, $P > 0.40$). This result was expected, since any tide-induced semidiurnal variations in the time series of capelin abundance would likely be obscured by the dominant diel cycle. To further explore the potential effects of tides on capelin emergence, the residuals of the regression of capelin abundance on irradiance were cross-correlated with tidal variables. These residuals included variations in capelin abundance that could not be explained by the diel fluctuations in irradiance, such as the daytime peaks observed on days 4 and 8 (Fig. 5a). A weak but statistically significant semidiurnal covariability was found between these residuals and water height, at a phase shift of 3 h (cross-correlation analysis: $r_{max} = 0.263$, $0.05 > P > 0.01$).Thus, once the dominant diel cycle was removed from the time series, capelin abundances tended to be higher than expected some 3 h after high water, during maximum ebb.

On the east coast of Newfoundland, larval capelin emergence has been related to the advection of surface waters by onshore winds (Frank and Leggett 1981a, 1983). In the present study, short-term fluctuations in emergence and wind force followed opposite diel cycles; nocturnal swarming of the larvae coinciding with low or null wind velocity (Fig. 2c). No positive correlation was found between diel or subdiel variations in larval capelin abundance and wind velocity from any of the azimuths considered (Table 4). However, the low-frequency trend in capelin abundance (Fig. 8a) was positively correlated with the low-frequency trend in the velocity

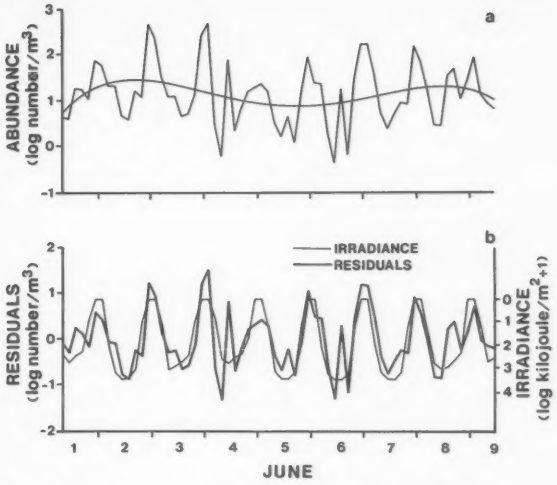

FIG. 8. (a) Time series of log-transformed larval capelin abundance in 1980. The trend was determined by fitting a polynomial function of time to the time series. (b) Stationary time series of larval capelin abundance and corresponding time series of solar irradiance. Note that the irradiance scale is inverted so that the trace represents "darkness intensity."

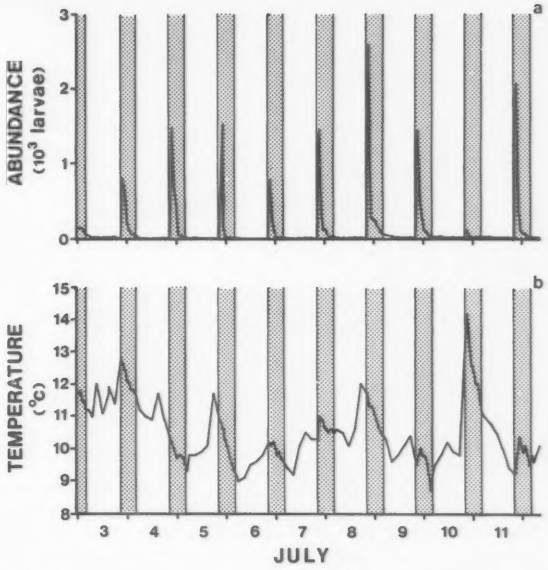

FIG. 9. (a) Abundance of larval capelin emerging from undisturbed sediments collected in Pointe-au-Père and incubated in the laboratory and (b) water temperature above the incubated sediments. Shaded areas indicate nighttime.

of southeasterly (Fig. 3e) and south-southeasterly winds (Table 4).

Emergence in the Laboratory

The emergence of larval capelin in the laboratory was precisely synchronized with the illumination cycle (Fig. 9a). Abundance was invariably maximum in the first collection (21:30) taken after the lights were turned off at 20:45. Direct observation using a dimmed flashlight showed that the larvae began to appear in the overlaying water during the first minutes of darkness. Abundance in the 21:30 collections varied from 788 to 2575 larvae, except on July 10 when excessive temperature may have thwarted emergence (Fig. 9b). These values corresponded to average emergence rates of 2322–7589 larvae$\cdot m^{-2}\cdot h^{-1}$ for the first 45 min of darkness (20:45–21:30). Emergence rates declined rapidly in the following hours of the night (Fig. 9a). Daytime collections (from 05:30 to 20:30) yielded few larvae, the rate of emergence ranging from 1.5 to 84 larvae$\cdot m^{-2}\cdot h^{-1}$ (average of 17.8 larvae$\cdot m^{-2}\cdot h^{-1}$).

Discussion

Capelin Emergence in the St. Lawrence Estuary

Both field and laboratory results showed that the emergence of larval capelin in the St. Lawrence estuary is dominated by a circadian rhythm. The laboratory experiment demonstrated that in the absence of tidal signal, most larvae spontaneously emerged from the sediment in the first 2 h following darkness. This suggests that larvae which hatch in daytime accumulated in the substratum and swarmed as soon as light intensity decreased.

Emergence in the field was predominantly nocturnal as well, but the concentration of emergence activity immediately after dusk was less evident than in the laboratory (compare Fig. 5a and 9a). This discrepancy could be related to a simple problem of aliasing: the sampling interval in the field was 3 h, and the first night collection was taken at 23:00, approximately 2 h after dusk. Thus, if the emerging larvae were rapidly carried away from the sampling area by currents, as is the case in coastal Newfoundland (Taggart 1986), the sampling hours in the field were possibly inadequate to detect maximum emergence occurring immediately after dusk.

Another discrepancy between laboratory and field results was the observation in situ of small but significant numbers of larvae in daytime. These occurred during maximum ebb current and generally on cloudy days (e.g. days 4 and 8). We suspect that increased turbidity during ebb, combined with heavy cloud cover, could trigger emergence in daytime by substantially attenuating the amount of light reaching the sediments. The demonstration of such an effect would require the direct measurement of incident light at the level of the spawning substrate during emergence. The advection by ebb tide of larvae recently emerged upstream of Saint-Irénée could also explain the presence in daytime of significant numbers of larvae in the vicinity of the intertidal spawning beds. The small but significant fraction of the residual variance in abundance that could not be explained by the dominant diel cycle but was related to the phase of the tide ($r^2_{max} = 0.069$) supports the latter interpretation.

In other respects, the semidiurnal tide appeared to have little influence on capelin emergence. If escapement from the spawning substrate at night depended on a precise tidal phase, nocturnal emergence would invariably occur during that tidal phase. The dominant diel pattern of emergence would then be modulated by the progressive shift of the tidal cycle relative to the diel cycle (~0.5 h per tidal cycle). Consequently, emergence would begin approximately 1 h later each night. A careful comparison of Fig. 2a and 5a reveals that nocturnal

TABLE 4. Correlation (r) between larval capelin abundance (log-normalized time series, stationary time series, and residuals of the regression against irradiance) and wind velocity from eight different azimuths. $*P < 0.05$; $**P < 0.01$; otherwise nonsignificant.

	N	NNE	NE	ENE	E	ESE	SE	SSE
				Wind velocity				
Abundance								
Log-normalized	−0.254*	0.236	−0.192	−0.138	−0.074	−0.006	0.108	0.209
Stationary	−0.234	−0.264**	−0.253*	−0.222	−0.174	−0.103	0.004	0.137
Residuals	−0.091	−0.056	−0.008	0.055	−0.077	−0.033	0.021	0.085
				Wind velocity (trend)				
Abundance (trend)								
Log-normalized	−0.174	0.042	0.181	0.279	0.360**	0.433**	0.484**	0.416**

emergence coincided with ebb during the first nights of sampling. Later, nocturnal emergence progressively became coincident with high tide. Thus, the timing of emergence was not modified by the phase shift of the tidal cycle over the diel cycle, indicating that the dominant circadian cycle of emergence was independant of tidal phase, at least as long as water covered the spawning grounds. During the period monitored, dusk never coincided with the exposure of the intertidal zone, and emergence always took place in water. We cannot reject the possibility that emergence which normally occurs immediately after dusk could be delayed until floodtide when the intertidal spawning beds are exposed at dusk.

At longer time scales, the low-frequency bimodal trend in the intensity of emergence could not be related to low-frequency tidal rhythms such as the neap-spring cycle in tidal amplitude. Given the weakness of the link between the timing or amplitude of emergence and either semidiurnal or fortnightly tides, we rejected the hypothesis that larval capelin emergence from the intertidal and subtidal zones of the St. Lawrence estuary is primarily synchronized to the strong tidal signals prevailing in the area.

On the eastern coast of Newfoundland, the active emergence of capelin larvae from intertidal beaches coincided with the advection by onshore winds of warm surface water masses (Frank and Leggett 1981a). Laboratory experiments confirmed that changes in the temperature or chemistry of the water, rather than mechanical disturbance of the substrate by waves, triggered emergence (Frank and Leggett 1983). The observation that swimming activity in the laboratory reached a maximum at the temperature of the incoming water masses (~8°C) suggested a direct link between temperature increases and the active emergence of the larvae (Frank and Leggett 1983).

In Saint-Irénée Bay, nocturnal swarming of larval capelin coincided with low or null wind velocity and could not be explained by wave-induced scouring of the intertidal flats. On the other hand, the low-frequency bimodal trend in capelin emergence and the trend in water temperature were both positively correlated with the trend in the velocity of onshore winds. These results indicated that while the timing of the diel cycle in emergence was unaffected by wind-related processes, low-frequency variations in the amplitude of nocturnal emergence were perhaps linked to some general increase in water temperature induced by onshore wind advection of warm surface waters as observed in Newfoundland. The trends in capelin emergence and water temperature were not significantly correlated, however ($r = 0.029$, $P = 0.819$).

A more plausible explanation is that the low-frequency bimodal trend in emergence reflected the successive maturation of distinct spawning waves. The interval between successive spawning waves and peaks in emergence (17–19 d) bracketed the mean time to hatching (18.2 d) predicted by the relationship between average temperature and time to hatching for capelin eggs incubated at an average temperature of 7.8°C (Frank and Leggett 1981b). This supports the hypothesis that emergence waves echoed spawning waves.

Adaptive Value of Littoral and Sublittoral Spawning in Capelin

The reproductive behaviour of typical intertidal spawners clearly exploits the special incubation conditions found in the upper reaches of the intertidal zone and the predictable return of high spring tides. The avoidance of low oxygen tension (Taylor and DiMichele 1983; Taylor 1984) or predators (Walker 1949) or the colonization of pools left by the receding spring tide to avoid loss of larvae to the open estuary (Taylor 1984) could yield survival advantages and thereby explain intertidal spawning in some species.

A study of egg predation by Frank and Leggett (1984) suggests that intertidal spawning in capelin is primarily "an effective means of reducing egg predation due both to a reduction in egg accessibility to most large fishes, especially gadoids, and to the thermal acceleration of egg development rates, which reduces the time that eggs are susceptible to predators."

An additional survival advantage related to littoral and shallow sublittoral spawning is the direct injection of the emerging larvae into productive surface waters. In the St. Lawrence estuary, emerging capelin larvae are rapidly carried by surface circulation towards the Gaspé current (Jacquaz et al. 1977; de Lafontaine et al. 1981, 1984a, 1984b; Fortier and Leggett 1982, 1983, 1985). Net plankton (15–200 μm), which comprises the food of early postlarvae, is particularly abundant in this area (Sévigny et al. 1979). The final resorption of the vitelline reserves of capelin larvae transported by the surface layer occurs at the head of the Gaspé current (Fortier and Leggett 1985) with the consequence that first feeding begins in a favourable prey field (Jacquaz et al. 1977).

On the east coast of Newfoundland, the emergence of the newly hatched larvae from the nearshore spawning beds may be delayed for several days until the advection by onshore winds of warmer surface waters (Frank and Leggett 1981a, 1982b, 1983). This dependence of emergence on an unpredictable wind regime increases the risk of early mortality (Frank and Leggett 1982a, 1983), and will tend to destabilize recrutement as suggested by the correlation found between onshore

wind frequency and year class strength (Leggett et al. 1984). The additional mortality risk related to the retention of the larvae in the beach sediments is apparently balanced by the advantages of synchronizing emergence with the inshore advection of warm surface water masses which are rich in plankton in the edible size range and contain relatively few predators (Frank and Leggett 1982b; Leggett 1986).

In both regions, the deposition of adherent eggs in the littoral or shallow sublittoral zone is followed by the direct emergence of the larval stages into the productive or potentially productive surface layer of annually recurring circulation patterns (Jacquaz et al. 1977; Frank and Leggett 1981a, 1983; Fortier and Leggett 1982, 1983, 1985; de Lafontaine et al. 1984a). Except for the shallow spawning of pelagic eggs, nearshore spawning remains the most economical means by which larval stages can colonize the surface layer, since no vertical migration is required. The alternative strategy of releasing pelagic eggs directly into the productive surface layers would expose the eggs to predation by pelagic invertebrates and planktivorous fish for up to 20 d before hatching occurred.

Producing pelagic eggs would also extend the duration of drift by about 20 d, thus increasing the probability of dispersion beyond areas of high productivity. For example, in the dispersion area of capelin spawned in the St. Lawrence estuary, the productive zones are located at the head of the Gaspé current (de Lafontaine et al. 1981; Therriault and Levasseur 1985). Pelagic eggs or larvae emerging directly in the Gaspé current would drift beyond these productive zones before hatching or the resorption of the vitelline reserves. The geographic distribution of spawning activity in the St. Lawrence estuary supports this view. Little spawning takes place along the Gaspé current itself. Rather, spawning is concentrated in the upper estuary, in the quadrilateral delineated by Tadoussac, Port-au-Persil, Rivière-du-Loup, and Rivière-aux-Vases (Fig. 1a) (Parent and Brunel 1976). The duration of drift from the area of maximum spawning to the area of maximum productivity corresponds closely to the duration of the yolk sac period (Fortier and Leggett 1985). Thus, littoral and sublittoral spawning allows the species to begin planktonic drift at a depth and in geographic locations that insure the transit of the passively drifting larvae (Fortier and Leggett 1982, 1983) through productive areas at the time of final yolk resorption (Jacquaz et al. 1977; de Lafontaine et al. 1984a; Fortier and Leggett 1985).

According to the developing paradigm on larval fish survival, mortality results primarily from predation during the embryonic and yolk sac stages and from starvation after the resorption of the yolk reserves (Blaxter and Hunter 1982; Hunter 1984; Leiby 1984; Lasker 1985; Leggett 1986). In a dynamic coastal circulation, getting the early postlarvae into productive areas at the right time while minimizing predation upon the vulnerable embryonic and yolk sac stages may be seen as a complex problem in ballistics. Littoral and sublittoral deposition of adherent eggs by capelin combines the advantages of reducing predation during the embryonic stage and of providing an optimal starting location in space for dispersion towards areas suitable for postlarval feeding and growth. In addition, the patterns of emergence from the nearshore spawning grounds are adapted to reduce predation at the onset of planktonic drift when the early yolk sac stages are densely aggregated (Frank and Leggett 1982b; this study). This interpretation of capelin reproductive strategy is consistent with the view that migratory and spawning behaviours of temperate fish are adapted to recurrent circulation patterns in ways that optimize the transport of the early stages towards areas suitable for growth and survival (e.g. Cushing 1972; Parent and Brunel 1976; Norcross and Shaw 1984; Leiby 1984).

Predator Avoidance and Stock Differentiation in Capelin

Nocturnal emergence has been reported in several other salmoniformes. For example, the larvae of the American smelt (*Osmerus mordax*), an anadromous osmerid that spawns demersal eggs in freshwater tributaries of the Estuary, emerge and drift at night (Ouellet and Dodson 1985). The larvae of the anadromous cisco (*Coregonus albula*) and whitefish (*C. laravaretus*) drift downstream at night, suggesting nocturnal hatching (Naesje and Jonsson 1986). Nocturnal emergence and drift of salmon fry have been widely documented (see Gustafson-Marjanen and Dowse 1983 for a detailed review).

Nocturnal emergence of salmoniform larvae is interpreted as an adaptation to avoid visual predators at the onset of drift when the larvae are densely aggregated (e.g. Solomon 1982; Gustafson-Marjenen and Dowse 1983; Naesje and Jonsson 1986). This adaptation could be particularly important for small capelin larvae which are poor swimmers. In the present study, the only visual predator identified with certainty was adult capelin. Feeding activity in adult capelin is depressed during spawning (Templeman 1948; Prokhorov 1965; Winters 1970; Vésin et al. 1981). Notwithstanding the unlikely explanation that cannibalism resulted solely from the concentration of the adults and the larvae in the codend of the nets, our results demonstrate that, even at night, mature or spent capelin can prey on the emerging larval stages to a variable but sometime important extent. Since adult capelin feed mainly during daytime (Vésin et al. 1981), the co-occurrence of adult schools and dense swarms of larvae emerging during the day could result in significant cannibalism. Nocturnal emergence would represent an adaptation to reduce this cannibalism and predation by other visual planktivores.

While very different, the emergence pattern of capelin larvae on the east coast of Newfoundland is also adapted to minimize predation. Escapement from the gravel beaches coincides with the sporadic advection by onshore winds of warm surface water masses containing significantly less predators than the cold-water masses associated with winds from other directions (Frank and Leggett 1982b). The active emergence of the larvae is triggered by abrupt changes in water temperature or chemistry caused by these coastal water mass exchanges (Frank and Leggett 1983). In the St. Lawrence estuary, the active emergence of the larvae from the intertidal flats is rather triggered by the onset of darkness. Thus, selection pressures acting to reduce predation at the beginning of planktonic drift in capelin favour the retention of a different behavioural response in each region. The existence of different behaviours suggests the existence of genetically distinct subpopulations or stocks.

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References

Akenhead, S. A., J. E. Carscadden, H. Lear, G. R. Lilly, and R. Wells. 1982. Cod–capelin interactions off northeast Newfoundland and Labrador, p. 141–148. *In* M. C. Mercer [ed.] Multispecies approaches to fisheries management advice. Can. Spec. Publ. Fish. Aquat. Sci. 59: 169 p.

Bailey, R. F. J., K. W. Able, and W. C. Leggett. 1977. Seasonal and vertical distribution and growth of juvenile and adult capelin (*Mallotus villosus*) in the St. Lawrence estuary and western gulf of St. Lawrence. J. Fish. Res. Board Can. 34: 2030–2040.

Blaxter, J. H. S., and J. R. Hunter. 1982. The biology of clupeoid fishes. Adv. Mar. Biol. 20: 1–223.

Carscadden, J. E. 1983. Capelin as a forage species: a review of selected studies. NAFO SCR Doc. 83/IX/72, Ser. No. N738: 7 p.

Cushing, D. H. 1972. The production cycle and the number of marine fish. Symp. Zool. Soc. Lond. 29: 213–232.

Davenport, J., and A. Stene. 1986. Freezing resistance, temperature and salinity tolerance in eggs, larvae and adults of capelin, *Mallotus villosus*, from Balsfjord. J. Mar. Biol. Assoc. U.K. 66: 145–157.

de Lafontaine, Y., M. I. El-Sabh, M. Sinclair, S. N. Messieh, and J.-D. Lambert. 1984a. Structure océanographique et distribution spatiotemporelle d'oeufs et de larves de poissons dans l'estuaire maritime et la partie ouest du golfe Saint-Laurent. Sci. Tech. Eau 17: 43–50.

de Lafontaine, Y., M. Sinclair, M. I. El-Sabh, C. Lassus, and R. Fournier. 1984b. Temporal occurrence of ichthyoplankton in relation to hydrographic and biological variables at a fixed station in the St. Lawrence estuary. Estuarine Coastal Shelf Sci. 18: 177–190.

de Lafontaine, Y., M. Sinclair, S. N. Messieh, M. I. El-Sabh, and C. Lassus. 1981. Ichthyoplankton distributions in the Northwest Gulf of St. Lawrence. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178: 185–187.

Fortier, L., and W. C. Leggett. 1982. Fickian transport and the dispersal of fish larvae in estuaries. Can. J. Fish. Aquat. Sci. 39: 1150–1163.

1983. Vertical migrations and transport of larval fish in a partially mixed estuary. Can. J. Fish. Aquat. Sci. 40: 1543–1555.

1985. A drift study of larval fish survival. Mar. Ecol. Prog. Ser. 25: 245–257.

Frank, K. T., and W. C. Leggett. 1981a. Wind regulation of emergence times and early larval survival in capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 38: 215–223.

1981b. Prediction of egg development and mortality rates in capelin (*Mallotus villosus*) from meteorological, hydrographic, and biological factors. Can J. Fish. Aquat. Sci. 38: 1327–1338.

1982a. Environmental regulation of growth rate, efficiency, and swimming performance in larval capelin (*Mallotus villosus*), and its application to the match/mismatch hypothesis. Can. J. Fish. Aquat. Sci. 39: 691–699.

1982b. Coastal water mass replacement: its effect on zooplankton dynamics and the predator–prey complex associated with larval capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 39: 991–1003.

1983. Survival value of an opportunistic life-stage transition in capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 40: 1442–1448.

1984. Selective exploitation of capelin (*Mallotus villosus*) eggs by winter flounder (*Pseudopleuronectes americanus*): capelin egg mortality rates, and contribution of egg energy to the annual growth of flounder. Can. J. Fish. Aquat. Sci. 41: 1294–1302.

Fridgeirsson, E. 1976. Observations on spawning behaviour and embryonic development of the Icelandic capelin. Rit Fiskideildar 5: 1–24.

Gustafson-Marjanen, K. I., and H. B. Dowse. 1983. Seasonal and diel patterns of emergence from the redd of Atlantic salmon (*Salmo salar*) fry. Can. J. Fish. Aquat. Sci. 40: 813–817.

Hunter, J. R. 1984. Inference regarding predators on the early life stages of cod and other fishes. *In* E. Dahl, D. S. Danielssen, E. Mockness, and P. Solemdal [ed.] The propagation of cod *Gadus morhua* L. Flod. Rapp. 1: 533–562.

Jacquaz, B., K. W. Able, and W. C. Leggett. 1977. Seasonal distribution, abundance, and growth of larval capelin (*Mallotus villosus*) in the St. Lawrence estuary and northwestern Gulf of St. Lawrence. J. Fish. Res. Board Can. 34: 2015–2029.

Jangaard, P. M. 1974. The capelin (*Mallotus villosus*). Bull. Fish. Res. Board Can. 186: 70 p.

Lasker, R. 1985. What limits clupeoid production? Can. J. Fish. Aquat. Sci. 42(Suppl. 1): 31–38.

Leggett, W. C. 1986. The dependence of fish larval survival on food and predator densities, p. 117–137. *In* S. Skreslet [ed.] The role of freshwater outflow in coastal marine ecosystems. NATO ASI Series Vol. G7. Springer-Verlag, Berlin.

Leggett, W. C., K. T. Frank, and J. E. Carscadden. 1984. Meteorological and hydrographic regulation of year-class strength in capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 41: 1193–1201.

Leiby, M. M. 1984. Life history and ecology of pelagic fish eggs and larvae, p. 121–140. *In* K. A. Steidinger and L. M. Walker [ed.] Marine plankton life cycle strategies. CRC Press, Inc., Boca Raton, FL.

Naesje, T. F., and B. Jonsson. 1986. Drift of cisco and whitefish larvae in a Norwegian river. Trans. Am. Fish. Soc. 115: 89–93.

Norcross, B. L., and R. F. Shaw. 1984. Oceanic and estuarine transport of fish eggs and larvae: a review. Trans. Am. Fish. Soc. 113: 153–165.

Ouellet, P., and J. J. Dodson. 1985. Tidal exchange of anadromous rainbow smelt (*Osmerus mordax*) larvae between a shallow tributary and the St. Lawrence estuary. Can. J. Fish. Aquat. Sci. 42: 1352–1358.

Parent, S., and P. Brunel. 1976. Aires et périodes de fraie du Capelan (*Mallotus villosus*) dans l'estuaire et le golfe Saint-Laurent. Trav. Pech. Que. 45: 1–46.

Pitt, T. K. 1958. Distribution, spawning and racial studies of the capelin, *Mallotus villosus* (Müller) in the offshore Newfoundland area. J. Fish. Res. Board Can. 15: 275–293.

Prokhorov, V. S. 1965. Ecology of the Barents Sea capelin (*Mallotus villosus* Müller) and the prospects for its commercial utilization. Tr. PINRO 19: 1–70. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. 813, 1967)

Sévigny, J.-M., M. Sinclair, M. I. El-Sabh, S. Poulet, and A. Coote. 1979. Summer plankton distributions associated with the physical and nutrient properties of the northwestern Gulf of St. Lawrence. J. Fish. Res. Board Can. 36: 187–203.

Solomon, D. J. 1982. Smolt migration in Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta* L.), p. 196–203. *In* E. L. Brannon and E. O. Salo [ed.]. Salmon and trout migratory behavior symposium. School of Fisheries, University of Washington, Seattle, WA.

Taggart, C. T. 1986. Mortality of larval capelin (*Mallotus villosus* Müller): environmental and density correlates during post-emergent dispersal. Ph. D. thesis, McGill University, Montréal, Qué.

Taylor, M. H. 1984. Lunar synchronization of fish reproduction. Trans. Am. Fish. Soc. 113: 484–493.

Taylor, M. H., and L. DiMichele. 1983. Spawning site utilization in a Delaware population of *Fundulus heteroclitus* (Pisces: Cyprinidintidae). Copeia 1983: 719–725.

Templeman, W. 1948. The life history of the capelin (*Mallotus villosus* O. F. Müller) in Newfoundland waters. Bull Nfld. Gov. Lab. No. 17: 151 p.

Therriault, J.-C., and M. Levasseur. 1985. Control of phytoplankton production in the lower St. Lawrence estuary: light and freshwater runoff. Nat. Can. 112: 77–96.

Vésin, J.-P., W. C. Leggett, and K. W. Able. 1981. Feeding ecology of capelin (*Mallotus villosus*) in the estuary and western Gulf of St. Lawrence and its multispecies implications. Can. J. Fish. Aquat. Sci. 38: 257–267.

Walker, B. W. 1949. Periodicity of spawning by the grunion. *Leuresthes tenuis*, an atherine fish. Ph.D. thesis, University of California, Los Angeles, CA.

Winters, G. H. 1969. Capelin (*Mallotus villosus*), p. 94–101. *In* F. E. Firth [ed.] Encyclopedia of marine resources. Van Nostrand Reinhold Co., New York, NY.

1970. Biological changes in coastal capelin from the over-wintering to the spawning condition. J. Fish. Res. Board Can. 27: 2215–2224.

Zwanenburg, K. C. T. 1982. The relationship of growth and larval mortality rates to food availability as measured *in situ* for larval yellow perch (*Perca flavescens*). M.Sc. thesis, McGill University, Montréal, Qué. 70 p.

Evaluation of Total Phosphorus as a Predictor of the Relative Biomass of Blue-green Algae with Emphasis on Alberta Lakes[1]

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Trimbee, A. M., and E. E. Prepas. 1987. Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. Can. J. Fish. Aquat. Sci. 44: 1337–1342.

The relative biomass of blue-green algae in freshwater (total dissolved solids < 500 mg·L^{-1}) Alberta lakes was consistently underestimated by two recent empirical models based on total nitrogen (TN), total phosphorus (TP), Secchi disc depth (SD), and depth of the mixed layer (Z_m). We regrouped the data used in these empirical models to eliminate the potential biases introduced by including data from lakes not in dynamic equilibrium and generated a new model based on TP. This new model accounted for 11% more of the variation in relative blue-green algal biomass than the original model based on TN, TP, SD, and Z_m and 21% more than the model based on TN to TP ratios and SD to Z_m ratios. This new model was also a much better predictor of the relative biomass of blue-green algae in Alberta lakes than the original models. In addition, for lakes in Alberta, TP was a much better predictor of total blue-green algal biomass than TN or the TN to TP ratio. Our analyses suggest that for large numbers of lakes, TP may be as good or better an indicator of relative and total biomass of blue-green algae than TN or TN to TP ratios.

La biomasse relative des algues bleu-vert (matières dissoutes totales < 500 mg·L^{-1}) des lacs d'eau douce de l'Alberta a, de façon constante, été sous-estimée par l'application de deux modèles empiriques récents reposant sur l'azote total (NT), le phosphore total (PT), la profondeur au disque de Secchi (PS) et la profondeur de la couche de mélange (Z_m). Les auteurs ont regroupé les données utilisées par ces modèles empiriques afin d'éliminer les biais pouvant être introduits par l'utilisation de données relatives à des lacs n'étant pas en équilibre dynamique, et ont produit un nouveau modèle basé sur le PT. Le nouveau modèle a permis d'expliquer 11 % de plus de la variation de la biomasse relative des algues bleu-vert par rapport au modèle antérieur reposant sur le NT, le PT, le PS et le Z_m, et 21 % de plus par rapport au modèle basé sur les rapports entre le NT et le PT et ceux entre le PS et le Z_m. Le nouveau modèle permettait aussi de prévoir avec beaucoup plus d'exactitude la biomasse relative des algues bleu-vert dans les lacs de l'Alberta, comparativement aux modèles antérieurs. De plus, dans le cas de ces lacs, le PT s'avérait un paramètre de prévision de la biomasse totale des algues bleu-vert de beaucoup supérieur au NT ou au rapport entre le NT et le PT. Les analyses effectuées par les auteurs portent à croire que, dans le cas d'un nombre important de lacs, le PT peut s'avérer un indicateur aussi valable, sinon supérieur, des biomasses relative et totale des algues bleu-vert que le NT ou les rapports entre le NT et le PT.

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In lakes, blue-green algae may produce unpleasant tastes, odors, and surface scums and are an unsatisfactory food source for many organisms higher in the trophic structure (Reynolds and Walsby 1975; Keating 1978; Horne 1979). Hypotheses to explain the success of blue-green algae include (1) the excretion of organic compounds that suppress growth of other algae (Murphy et al. 1976; Keating 1978), (2) the minimization of mortality through an immunity to grazing by zooplankton (Porter 1973; Kalff and Knoechel 1978), and (3) superior uptake kinetics for inorganic carbon (King 1970; Shapiro 1973, 1984).

Recently, the competitive advantage of blue-green algae under conditions of nitrogen limitation (Schindler 1977; Flett et al. 1980) has been emphasized. Smith (1983) reported that blue-green algal blooms occurred only, but not necessarily, when epilimnetic total nitrogen (TN) to total phosphorus (TP) ratios by weight were below 29:1 and that blue-green algae were rare when TN to TP ratios exceeded 29:1. Recently, Smith (1986) suggested that TN, TP, and light availability all have an influence on the relative biomass of blue-green algae; thus, the relative biomass of blue-green algae would be high when the TN to TP ratio was high if light availability was low (Table 1, Eq. S1 and S2). Although the sample size used in Smith's (1986) equations to predict the relative biomass of blue-green algae was large (n = 107), the data were from only 20 lakes. We see three potential problems with Smith's (1986) data set as he used it.

(1) When several years' data were available from a single lake, each year was treated separately. Many of the lakes included in the data set were undergoing rapid changes in nutrient loading, e.g. Lake Trummen (Bjork 1979) and Lake Washington (Edmondson and Lehman 1981). The use of lakes not in dynamic equilibrium could introduce a serious bias.

(2) In some cases, information from different stations in heterogeneous lakes such as Hjälmaren, Sweden (Willen 1975), was treated as separate data. Phytoplankton produced in nutrient-rich areas of these lakes could be transported through advection to areas of the lake where nutrients were low.

(3) Data from three limnocorral nutrient manipulation experiments were used. These systems were probably not in dynamic equilibrium.

[1]Contribution to the Narrow Lake project, based at the Meanook Biological Station.

TABLE 1. Smith's models for the prediction of relative biomass of blue-green algae (Eq. S1 and S2 from Smith 1986) and total blue-green algal biomass (Eq. S3, S4, and S5 from Smith 1985). TN = total nitrogen ($\mu g \cdot L^{-1}$), TP = total phosphorus ($\mu g \cdot L^{-1}$), TN:TP = TN to TP ratio, SD = secchi disc depth (m), Z_m = depth of the mixed layer (m), SD:Z_m = ratio of the Secchi disc depth to the depth of the mixed layer, $\bar{z}$ = mean depth (m), %BG = relative proportion of blue-green algae, logit (%BG) = $\log_{10}$ (%BG/(100−%BG)).

(S1)	logit (%BG) = 2.358 − 1.297 log TN + 0.692 log TP − 2.058 log SD + 0.538 log Z_m,	$r^2 = 0.52$
(S2)	logit (%BG) = 1.096 − 1.604 log TN:TP − 1.119 log SD:Z_m,	$r^2 = 0.42$
(S3)	log BG biomass ($mg \cdot L^{-1}$) = −0.142 + 0.596 log TP −0.963 log $\bar{z}$,	$r^2 = 0.795$
(S4)	log BG biomass ($mg \cdot L^{-1}$) = −2.994 + 1.244 log TN −0.751 log $\bar{z}$,	$r^2 = 0.794$
(S5)	log BG biomass ($mg \cdot L^{-1}$) = −2.203 − 0.835 log TN:TP −1.336 log $\bar{z}$,	$r^2 = 0.765$

TABLE 2. Data on Alberta lakes. TP = total phosphorus, TN = total nitrogen, $\bar{z}$ = mean depth, Z_m = depth of the mixed layer, SD = Secchi disc depth, %BG = relative biomass of blue-green algae, and BGB = blue-green algal biomass. All data are average spring through summer values.

Lake	Year	TP ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	$\bar{z}$ (m)	Z_m (m)	SD (m)	%BG	BGB ($mg \cdot L^{-1}$)
Bourque	1981	17	800	8.3	9.0	3.2	7	0.94
Ethel	1983	20	715	6.6	8.0	3.0	23	0.45
Ethel	1981	21	818	6.6	8.0	3.0	38	1.08
Hilda	1981	22	1217	6.2	8.0	2.0	34	0.42
Moore	1981	25	1051	8.3	11.0	4.1	10	2.22
Island S.	1983	26	1073	5.2	5.2	3.4	18	0.36
Battle	1983	33	675	5.5	5.5	3.6	38	1.03
Amisk N.	1984	33	1084	10.7	7.0	2.8	35	1.76
Buck	1983	38	770	6.2	6.2	3.4	57	1.35
Baptiste N.	1983	47	1148	5.9	5.9	1.0	24	2.11
Baptiste S.	1983	52	1160	12.7	11.0	1.3	51	5.68
Pine	1979	53	1370	6.0	6.0	3.3	26	0.55
Tucker	1979	59	995	2.9	2.9	0.7	88	28.84
Wizard	1979	61	1460	5.0	5.0	1.5	55	7.53
LaBiche	1979	78	1050	8.4	8.4	2.2	81	7.78
Isle	1983	93	1073	4.5	4.5	2.1	55	2.69
Nakamun	1983	107	1574	4.5	4.5	0.8	72	4.04
Nakamun	1979	144	2240	4.5	4.5	2.5	87	12.30
LaNonne	1979	192	2300	7.6	10.0	1.6	71	15.85

Smith (1985) used a subset of the data used in his 1986 paper to develop three equations to predict total blue-green algal biomass. With data from only three limnocorrals and three lakes, but several data points from each lake so that n = 29, Smith found that mean depth ($\bar{z}$) combined with TP concentrations, TN concentrations, or TN to TP ratios were all good predictors of blue-green algal biomass (Table 1, Eq. S3, S4, and S5). TP was highly correlated with TN in this small data set (r = 0.96). These three models were statistically indistinguishable from one another (Smith 1985). The data used in these models have the same potential problems discussed above; the data were from lakes and limnocorrals not in dynamic equilibrium.

In Alberta, despite relatively high TN to TP ratios (12 to 55), blue-green algae are important, comprising 7−88% of the average spring through summer phytoplankton biomass (Table 2). Many of these lakes are highly productive (Riley and Prepas 1984). However, unlike many of the eutrophic lakes used in Smith's (1985, 1986) models, these Alberta lakes are naturally eutrophic and have not been exposed to recent dramatic increases in cultural nutrient loading. None of the Alberta lakes have had nutrient removal schemes implemented.

The objective of this study was to evaluate whether TP is as good or better a predictor than TN or TN to TP ratios of the relative blue-green algal biomass in lake phytoplankton with reference to Alberta lakes. We began by regrouping the data used in Smith's (1986) models to eliminate the potential biases discussed above and generated a new model based on TP. We then tested Smith's (1986) original empirical models (Table 1, Eq. S1 and S2), and this new model that we generated from his data, on new data from Alberta lakes. In addition, we evaluated whether TP was as good or better a predictor than TN or TN to TP ratios of total blue-green algal biomass in Alberta lakes.

Materials and Methods

Information on spring through summer euphotic or epilimnetic blue-green algal biomass, total phytoplankton biomass, TP, TN, Secchi disc depth (SD), mixed layer depth (Z_m), and mean depth ($\bar{z}$) was obtained for 16 Alberta lakes (Table 2, $n = 19$). Data for Amisk, Bourque, Ethel, Hilda, Moore, and Tucker lakes were obtained from Prepas and Trew (1983), Trew et al. (1981), and A. M. Trimbee and E. E. Prepas (unpubl. data). These lakes were sampled from 2 to 11 times ($\bar{x} = 6$) over the spring and summer period. Analyses of TN (calculated as the sum of total Kjeldahl nitrogen (TKN), NO_3^-, and NO_2^--N) and TP were carried out as outlined in Prepas and Trew (1983). Data for Baptiste N., Baptiste S., Battle, Island, Isle, LaBiche, LaNonne, Nakamun, Pine, and Wizard are from P. Mitchell (Alberta Environment, unpubl. data). These lakes were sampled six times in the spring and summer. Algal biomass estimates were obtained from R. S. Anderson, D. Beliveau, and A. Furnell (Alberta Environment, unpubl. data). Cell volume estimates were converted to algal biomass on the assumption that 1 $mm^3 \cdot m^{-3}$ is equivalent to 1 mg wet $wt \cdot m^{-3}$ (Vollenweider 1969). Data from two different years for Ethel and Nakamun lakes and from one year for two different basins of Baptiste Lake were treated as independent data points. The external nutrient inputs to these lakes are relatively constant from year to year.

To bring the data as close as possible to a normal distribution, the relative blue-green algal biomass was transformed:

$$(1)\quad \text{BG index} = \ln(\%BG/(100 - \%BG))$$

where %BG is the percent of total phytoplankton biomass made up by blue-green algae. The BG index can range from −4.595 (%BG = 1) to 4.595 (%BG = 99). The remaining data were $\log_{10}$-transformed to stabilize the variance, to linearize the data, and to distribute the data more evenly.

To evaluate the relative predictive abilities of Smith's (1986) original models (Table 1, Eq. S1 and S2) and the new model based on TP on data from Alberta lakes, we compared the residual mean squares (RMS). The RMS for each model was calculated as follows:

$$(2)\quad \text{RMS} = \sum_{i=1}^{n} (\text{pred}_i - \text{obs}_i)^2/\text{df}$$

where pred_i is the predicted relative blue-green algal biomass and obs_i is the observed relative blue-green algal biomass for each lake (i) and df is the degrees of freedom. For each model, df was defined as the number of data points minus 1 minus the number of independent variables in the model. All statistical analyses were done with Minitab statistical software on the University of Alberta Amdahl computer.

General Characteristics of the Study Lakes

The Alberta lakes are located in the boreal mixed-wood and lower foothills transition biomes in central Alberta, within a 300-km radius of Edmonton. Most of the Alberta lakes are in forested drainage basins, with anywhere from none to a moderate portion of the drainage basin used for grazing or crops. In this region, annual precipitation and lake evaporation both range from 300 to 500 mm (Fisheries and Environment Canada 1978). These lakes are freshwater (total dissolved solids < 500 $mg \cdot L^{-1}$), are not heavily colored (5–25 mg $platinum \cdot L^{-1}$), and most are not very turbid (0.1–22 Jackson turbidity units) (Prepas and Trew 1983).

Results

To examine the potential biases in Smith's (1986) models to predict the relative biomass of blue-green algae, his data (originally $n = 107$) were regrouped to form 36 data points (Table 3). The following criteria were applied.

(1) When data were available for several years for a lake undergoing rapid changes in nutrient loading, we averaged data for individual years in 2- to 5-yr groupings. The number of years grouped depended on how rapidly nutrient levels were changing; the number of years grouped was greater when nutrient levels were changing rapidly.

(2) Information from several stations within a lake in a given year was averaged.

(3) Data from limnocorrals ($n = 3$) were excluded.

With the 36 regrouped data points, the relative biomass of blue-green algae was best estimated by SD or TP (Fig. 1):

$$(3)\quad \text{BG index} = 0.10 - 3.37 \log \text{SD}, \quad r^2 = 0.64$$

$$(4)\quad \text{BG index} = -5.00 + 2.62 \log \text{TP}, \quad r^2 = 0.63.$$

TP (Eq. 4) alone accounted for 10% more of the variation than Smith's (1986) models with four variables (Table 1, Eq. S1) and 20% more than his model with two variables (Table 1, Eq. S2). TP and SD were much better predictors of the relative contribution of blue-green algae than TN or TN to TP ratios:

$$(5)\quad \text{BG index} = -9.53 + 3.00 \log \text{TN}, \quad r^2 = 0.43$$

$$(6)\quad \text{BG index} = 5.21 - 4.54 \log \text{TN:TP}, \quad r^2 = 0.44.$$

The model that we generated from Smith's (1986) regrouped data, based on TP (Eq. 4), was also a much better predictor of the relative biomass of blue-green algae in Alberta lakes than Smiths's (1986) original models based on TN, TP, and light availability (Table 4). The residual mean square was 382 for our model based on TP (Eq. 4) as compared with 1358 for Eq. S1 and 887 for Eq. S2. Smith's (1986) models consistently underestimated the relative biomass of blue-green algae in Alberta lakes.

The following models to predict total blue-green algal biomass were developed from our data set of Alberta lakes:

$$(7)\quad \log \text{BG biomass } (mg \cdot L^{-1}) = -1.92 + 1.34 \log \text{TP}, \quad r^2 = 0.67$$

$$(8)\quad \log \text{BG biomass } (mg \cdot L^{-1}) = -6.46 + 2.22 \log \text{TN}, \quad r^2 = 0.43$$

$$(9)\quad \log \text{BG biomass } (mg \cdot L^{-1}) = 2.76 - 1.76 \log \text{TN:TP}, \quad r^2 = 0.54.$$

Tucker Lake was not included in the above analyses, since the total blue-green algal biomass in Tucker Lake was disproportionately high in relation to TP, TN, or TN to TP ratios. In contrast with Smith's (1985) models to predict total blue-green algal biomass, but in agreement with our findings when we reworked his 1986 data set to predict the relative biomass of blue-green algae, TP accounted for a much greater amount of

TABLE 3. Regrouped data from Smith (1986). TP = total phosphorus, TN = total nitrogen, SD = Secchi disc depth, Z_m = depth of the mixed layer, $\bar{z}$ = mean depth, and %BG = relative biomass of blue-green algae.

Lake	Year	TP ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)	SD (m)	Z_m (m)	$\bar{z}$ (m)	%BG
Washington	1963–65	53	539	1.23	10.0	32.9	85.4
	1966–68	28	514	1.57	10.0	32.9	77.2
	1969–71	15	336	2.82	10.0	32.9	39.6
	1972–74	13	280	3.24	10.0	32.9	30.7
	1975–77	10	244	5.95	10.0	32.9	10.8
	1978–79	11	200	7.26	10.0	32.9	11.5
Tahoe	1969–73	3	61	28.30	15.0	313.0	1.0
Loch Leven	1968–71	86	1406	1.22	3.9	3.9	40.6
Norvikken	1969–72	239	2875	0.69	6.2	5.4	85.3
	1973–76	134	1900	0.82	6.1	5.4	90.2
Ontario	1972	18	356	4.42	16.3	90.0	29.6
Trummen	1968–69	728	6319	0.19	1.1	1.1	93.9
	1970–73	151	2166	0.71	1.7	1.7	34.4
	1974–78	73	1462	0.64	1.8	1.8	75.4
Hjälmaren[a]	1966–71	148	1567	0.32	2.9	2.9	63.3
Vänern	1972–73	8	700	4.00	20.0	28.0	1.0
Huron	1972	37	608	1.06	6.0	6.0	44.8
Mälaren[b]	1969	58	1170	1.59	8.5	13.7	26.2
	1970	50	1543	1.91	11.1	13.7	24.4
	1971	43	1037	2.34	10.3	13.7	10.0
	1972	47	943	2.24	10.7	13.7	20.4
Heart	1973	91	1202	1.56	4.0	4.6	85.5
Michigan	1976	8	392	7.63	16.0	85.0	15.6
George	1967–72	220	2550	0.31	2.4	2.4	80.0
Kinneret	1969–72	7	458	3.75	23.0	25.0	2.5
Slapy	1961	38	1429	2.00	32.5	20.6	59.6
	1963	35	1779	2.00	5.0	20.6	16.4
Vättern	1972–73	5	500	10.00	35.0	35.0	1.0
Uttran	1972–74	72	1412	1.52	8.0	6.4	50.7
Langvatn	1975	18	178	3.60	5.0	18.3	3.0
Ostra R.	1975–81	251	2681	0.53	6.0	15.0	62.6
Vastra R.	1975–81	207	2516	0.47	5.0	5.0	71.2
Saginaw B.	1974	29	618	1.84	8.7	8.7	23.7
	1975	28	686	2.10	8.7	8.7	15.8
Sodra B.	1973–76	1025	3975	0.44	2.3	2.3	68.0
Blacken[c]	1965–68	50	1077	1.52	11.0	11.0	18.1

[a]Three stations combined.
[b]Four stations combined.
[c]One bay in Mälaren averaged over 4 yr.

the variance than TN or TN to TP ratios. Also, in the prairie lakes, TP was not as highly correlated with TN ($r = 0.79$) as it was in Smith's data ($r = 0.96$).

Discussion

Smith (1986) stated that the TN to TP ratio in water from the epilimnion and light availability control the relative blue-green algal biomass in lake phytoplankton. Results from this study suggest that the degree of eutrophy, as estimated by TP levels or SD, is the dominant factor and that decreases in TP should result in decreases in the relative biomass of blue-green algae. In addition, for Alberta lakes, unlike the lakes in Smith's (1985) model to predict the total blue-green algal biomass, TP was a much better predictor of total blue-green algal biomass than TN or TN to TP ratios. Although it is generally accepted that blue-green algae are more abundant in eutrophic lakes (Reynolds and Walsby 1975; Healey 1982; Shapiro 1984), our model (Eq. 4) is the first relating TP to the relative biomass of blue-green algae. There are many examples in the literature where decreases in phosphorus loading have resulted in decreases in blue-green algae (e.g. Dillon et al. 1978; Bjork 1979; Sze 1980). In contrast, Reckhow and Simpson (1980) concluded that the ratio of blue-green algae to green algae was not related to TP levels for a collection of lakes sampled for the U.S. Environmental Protection Agency National Eutrophication Survey Program. However, the authors pointed out that their selection criteria for lakes favored the inclusion of lakes impacted by cultural sources, notably sewage treatment plants, and suggested that the effects of TP were nullified.

The increase in the relative biomass of blue-green algae with eutrophication is potentially a result of both increases in TP and associated changes such as increased oxygen depletion rates (Cornett and Rigler 1980), decreased TN to TP ratios (Forsberg 1979), and decreased light availability. The development of anoxic conditions over lake sediments favors the development of blue-green algae through enhanced recruitment of benthic overwintering blue-green algal colonies or

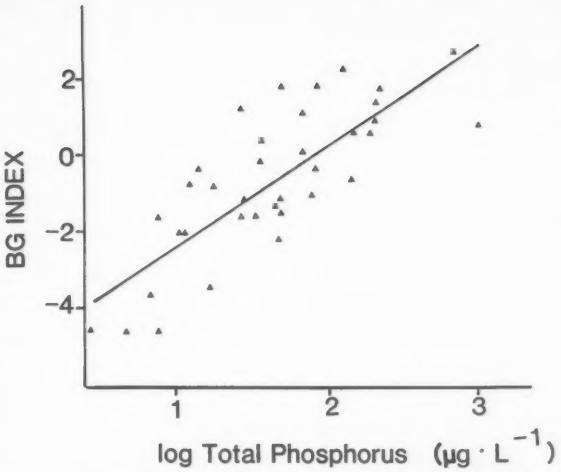

FIG. 1. Regression of relative blue-green algal biomass (BG index) on total phosphorus for the regrouped data from Smith (1986). Statistics required for the construction of confidence limits for the regression (Sokal and Rohlf 1969) are $S^2_{y \cdot x} = 1.433$, $\Sigma x^2 = 12.2037$, $S_b = 0.3427$, $\bar{X} = 1.6475$.

TABLE 4. Comparison of observed relative blue-green algal biomass in Alberta lakes with predictions from Smith's (1986) original models (Eq. S1 and S2 in Table 1) and from the new model (Eq. 4) based on total phosphorus, generated from regrouping Smith's (1986) data set. %BG = relative blue-green algal biomass.

		%BG			
			Predictions		
Lake	Year	Observed	Eq. S1	Eq. S2	Eq. 4
Bourque	1981	7	8	8	14
Ethel	1983	23	10	11	17
Ethel	1981	38	9	10	18
Hilda	1981	34	12	9	18
Moore	1981	10	5	9	21
Island S.	1983	18	5	5	22
Battle	1983	38	9	14	26
Amisk	1984	35	9	11	26
Buck	1983	57	10	16	30
Baptiste N.	1983	24	48	35	35
Baptiste S.	1983	51	44	48	37
Pine	1979	26	6	12	38
Tucker	1979	88	65	40	41
Wizard	1979	55	24	23	42
LaBiche	1979	81	26	46	49
Isle	1983	55	23	37	54
Nakamun	1983	72	59	55	58
Nakamun	1979	87	10	23	66
LaNonne	1979	71	33	64	73

filaments into the phytoplankton (Reynolds et al. 1981; Trimbee and Harris 1984). In addition, anoxic conditions over lake sediments may result in increased TP levels in the epilimnion (Prepas and Vickery 1984). In some lakes, blue-green algal biomass increases as the proportion of lake sediments covered by anoxic water increases, even without increases in epilimnetic TP (Trimbee and Prepas 1987). The relative biomass of blue-green algae is especially high in lakes where the development of anoxic conditions over lake sediments results in almost immediate increases in epilimnetic TP (Trimbee and Prepas 1987). Although it is clear from whole-lake and limnocorral experiments that low TN to TP ratios favor nitrogen-fixing blue-green algae (Findlay and Kling 1975; Lean and Charlton 1976; Schindler 1977; Barica et al. 1980; Flett et al. 1980; Nalewajko and Lean 1980), it is not clear that it is because of low TN to TP ratios that blue-green algae, which may or may not fix nitrogen, become dominant in eutrophic systems. Smith (1986) suggested that increases in the TN to TP loading ratio may not be enough to minimize the relative biomass of blue-green algae, and also recommended that total phosphorus loading be decreased. TN to TP ratios may be more important in lakes undergoing dramatic changes in nutrient loading than in those with relatively constant annual inputs.

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References

BARICA, J., H. KLING, AND J. GIBSON. 1980. Experimental manipulation of algal bloom composition by nitrogen addition. Can. J. Fish. Aquat. Sci. 37: 1175–1183.

BJORK, S. 1979. Lake management studies and results at the Institute of Limnology in Lund. Arch. Hydrobiol. Beih. Ergebn. Limnol. 13: 31–55.

CORNETT, J., AND F. H. RIGLER. 1980. The areal hypolimnetic oxygen deficit: an empirical test of the model. Limnol. Oceanogr. 25: 672–679.

DILLON, P. J., K. H. NICHOLLS, AND G. W. ROBINSON. 1978. Phosphorus removal at Gravenhurst Bay, Ontario: an 8 year study on water quality changes. Int. Ver. Theor. Angew. Limnol. Verh. 20: 263–271.

EDMONDSON, W. T., AND J. T. LEHMAN. 1981. The effect of changes in the nutrient income on the condition of Lake Washington. Limnol. Oceanogr. 26: 1–29.

FINDLAY, D. L., AND H. J. KLING. 1975. Seasonal successions of phytoplankton in seven lake basins in the Experimental Lakes Area, northwestern Ontario following artificial eutrophication. Fish. Mar. Res. Dev. Serv. Tech. Rep. 513: 53 p.

FISHERIES AND ENVIRONMENT CANADA. 1978. Hydrological atlas of Canada. Ministry of Supply and Services Canada, Ottawa, Ont. 267 p.

FLETT, R. J., D. W. SCHINDLER, R. D. HAMILTON, AND N. E. R. CAMPBELL. 1980. Nitrogen fixation in Canadian Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 37: 494–505.

FORSBERG, C. 1979. Tie physiologischen Grundlangen der Gewässer-Eutrophierung. Wasser Abwassen Forschung. 12: 40–45.

HEALEY, F. P. 1982. Phosphate, p. 105–125. *In* N. G. Carr and B. A. Whitton [ed.] The biology of cyanobacteria. Botanical monographs. Vol. 19. Blackwell, Oxford.

HORNE, A. J. 1979. Management of lakes containing N_2 fixing blue-green algae. Arch. Hydrobiol. Beih. Ergebn. Limnol. 13: 133–144.

KALFF, J., AND R. KNOECHEL. 1978. Phytoplankton and their dynamics in oligotrophic and eutrophic lakes. Annu. Rev. Ecol. Syst. 9: 475–495.

KEATING, K. I. 1978. Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science (Wash., DC) 199: 971–973.

KING, D. 1970. The role of carbon in eutrophication. J. Water Pollut. Control Fed. 42: 2035–2051.

LEAN, D. R. S., AND M. N. CHARLTON. 1976. A study of phosphorus kinetics in a lake ecosystem. p. 283–294. *In* J. O. Nriagu [ed.] Environmental biogeochemistry. Vol. 1: Carbon, nitrogen, phosphorus, sulfur and

selenium cycles. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.

MURPHY, T. P., D. R. S. LEAN, AND C. NALEWAJKO. 1976. Blue-green algae: their excretion of iron-selective chelators enables them to dominate other algae. Science (Wash., DC) 192: 900–902.

NALEWAJKO, C., AND D. R. S. LEAN. 1980. Phosphorus, p. 235–258. *In* I. Morris [ed.] The physiological ecology of phytoplankton. Blackwell, Oxford.

PORTER, K. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature (Lond.) 244: 179–180.

PREPAS, E. E., AND D. O. TREW. 1983. Evaluation of the phosphorus–chlorophyll relationship for lakes off the Precambrian Shield in western Canada. Can. J. Fish. Aquat. Sci. 40: 27–35.

PREPAS, E. E., AND J. VICKERY. 1984. Seasonal changes in total phosphorus and the role of internal loading in western Canadian lakes. Verh. Int. Ver. Limnol. 22: 303–308.

RECKHOW, K. W., AND J. T. SIMPSON. 1980. An empirical study of factors affecting blue-green versus non blue-green algal dominance in lakes. U.S. Natl. Tech. Inf. Serv. Publ. PB 80-169311: 108 p.

REYNOLDS, C. S., G. H. M. JAWORSKI, H. A. CMIECH, AND F. LEEDALE. 1981. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kutz. emend Elenkin. Philos. Trans. R. Soc. Lond. B 292: 419–477.

REYNOLDS, C. S., AND A. E. WALSBY. 1975. Water-blooms. Biol. Rev. 50: 437–481.

RILEY, E. T., AND E. E. PREPAS. 1984. Role of internal phosphorus loading in two shallow, productive lakes in Alberta, Canada. Can. J. Fish. Aquat. Sci. 41: 845–855.

SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. Science (Wash., DC) 195: 260–262.

SHAPIRO, J. 1973. Blue-green algae: why they become dominant. Science (Wash., DC) 179: 382–384.

1984. Blue-green algal dominance in lakes: the role and management significance of pH and CO_2. Int. Rev. Gesamten. Hydrobiol. 69: 765–780.

SMITH, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science (Wash., DC) 221: 669–671.

1985. Predictive models for the biomass of blue-green algae in lakes. Water Resour. Bull. 21: 433–439.

1986. Light and nutrient effects on the relative biomass of blue-green algae in lake phytoplankton. Can. J. Fish. Aquat. Sci. 43: 148–153.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry: the principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco, CA. 776 p.

SZE, P. 1980. Seasonal succession of phytoplankton in Onondaga Lake, New York (USA). Phycologia 19: 54–59.

TREW, D. O., E. I. YONGE, AND R. P. KAMINSKI. 1981. A trophic assessment of nine lakes in the Cold Lake Region. Alberta Environment Interim Report. Pollution Control Division, Water Quality Control Branch. 70 p.

TRIMBEE, A. M., AND G. P. HARRIS. 1984. Phytoplankton dynamics of a small reservoir: use of sedimentation traps to quantify the loss of diatoms and recruitment of summer bloom-forming blue-green algae. J. Plankton Res. 5: 897–918.

TRIMBEE, A. M., AND E. E. PREPAS. 1987. The effect of oxygen depletion on the timing and magnitude of blue-green blooms. Verh. Int. Ver. Limnol. 23. (In press)

VOLLENWEIDER, R. A. 1969. Measuring primary production in aquatic environments. IBP Handbook 12. Blackwell, Oxford. 213 p.

WILLEN, T. [ED.] 1975. Vättern, Vänern, Mälaren, Hjälmaren, en Oversikt. Statens naturvardsverk publication 1976: 121 p.

Laboratory Apparatus for Studying the Behaviour of Organisms in Vertically Stratified Waters

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Birtwell, I. K., and G. M. Kruzynski. 1987. Laboratory apparatus for studying the behaviour of organisms in vertically stratified waters. Can. J. Fish. Aquat. Sci. 44: 1343–1350.

An aquarium system is described which was designed to examine the locomotor activity of juvenile salmon under simulated estuarine conditions. A stable, vertically stratified water column can be established and dissolved oxygen, salinity, water velocity, and light intensity varied during operation under continuous-flow or recirculating conditions. Examples of stratification stability and application to studies of fish behaviour in relation to hypoxia are also presented.

Les auteurs décrivent un aquarium conçu pour l'étude des activités locomotrices des saumons juvéniles en conditions estuariennes simulées. Le dispositif permet d'obtenir une masse d'eau à stratification verticale stable et de varier la teneur en oxygène dissous, la salinité, la vitesse d'écoulement et l'intensité lumineuse en modes d'écoulement continu ou de recirculation. Des exemples de stabilité de la stratification et de l'application du système à des études de comportement des poissons en régime d'hypoxie sont aussi présentés.

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In recent years a number of studies have focussed on the role of estuaries in the ecology of Pacific salmon (genus *Oncorhynchus*). With the increasing intrusion of man's activities into these areas, an understanding of how salmon utilize such transition zones is fundamental to sound fisheries and environmental management. Researchers have generally addressed aspects of juvenile salmon residency (Reimers 1973; Mason 1974; Healey 1979, 1980; Levy and Northcote 1981, 1982; Levings et al. 1983), distribution and abundance (Beak Consultants Ltd. 1981; Gordon and Levings 1984; Macdonald 1984), and trophic relationships (Healey 1982). However, much less emphasis has been placed on studying the behaviour of juvenile salmonids in estuaries, an aspect which is inherently linked to survival (Mace 1983; Macdonald et al. 1985; Piercey et al. 1985).

In British Columbia many estuaries are vertically stratified, with a well-defined halocline close to the surface. Wastes discharged into rivers or directly into the freshwater lens may be confined there, due to the vertical density stratifications, and thus impose constraints upon resident and transient biota. Investigations by Birtwell (1977), Birtwell and Harbo (1980), Birtwell et al. (1983), and McGreer and Vigers (1983) demonstrated how the characteristic surface water orientation behaviour of juvenile salmonids in estuaries may be changed in response to the discharge of pulp mill effluent. Thus there is a potential conflict between the need to dispose of wastes and the requirements of aquatic organisms to use shallow surface waters.

Although salmonid behaviour can be observed in clear estuarine waters in relation to physico-chemical parameters (Macdonald et al. 1987), this is impossible in turbid waters such as those of the Fraser River estuary. To facilitate research on the behaviour of juvenile salmon and other organisms, we developed a water column simulator (WCS) that permits controlled manipulation of several physico-chemical conditions thought to influence salmon behaviour in vertically stratified waters. A major feature of the apparatus is the maintenance of a well-defined halocline between adjacent horizontally flowing water masses, a hydrographic feature which has been associated with fish orientation during migration in response to olfactory cues (McInerney 1964; Brawn 1982; Westerberg 1982, 1984), and osmoregulation (Iwata and Komatsu 1984). The apparatus provides an opportunity to examine organisms in the vertical plane, a technique which has not been widely applied in fish behaviour studies (Brett 1952; Olla et al. 1967; Hurley and Woodall 1968; Scherer 1976; Purcell and Shrode 1983). It differs, therefore, from the majority of other systems used in studies which have employed steep or shallow gradients in the horizontal plane (McCauley 1977; Larrick et al. 1978; Giattina and Garton 1983; Rand 1985).

Our objective here is to provide a description of the apparatus at its current stage of development, present data that demonstrate the maintenance of stable vertically stratified conditions between adjacent horizontally flowing water masses, and to exemplify its application to research on salmonid behaviour.

Apparatus Components

The apparatus comprises an aquarium, provided with a water delivery, mixing, and regulation system, novel lighting, continuous physico-chemical monitoring, and time-lapse image recording. Relative to other systems used to study fish under stratified conditions, the WCS offers greater flexibility

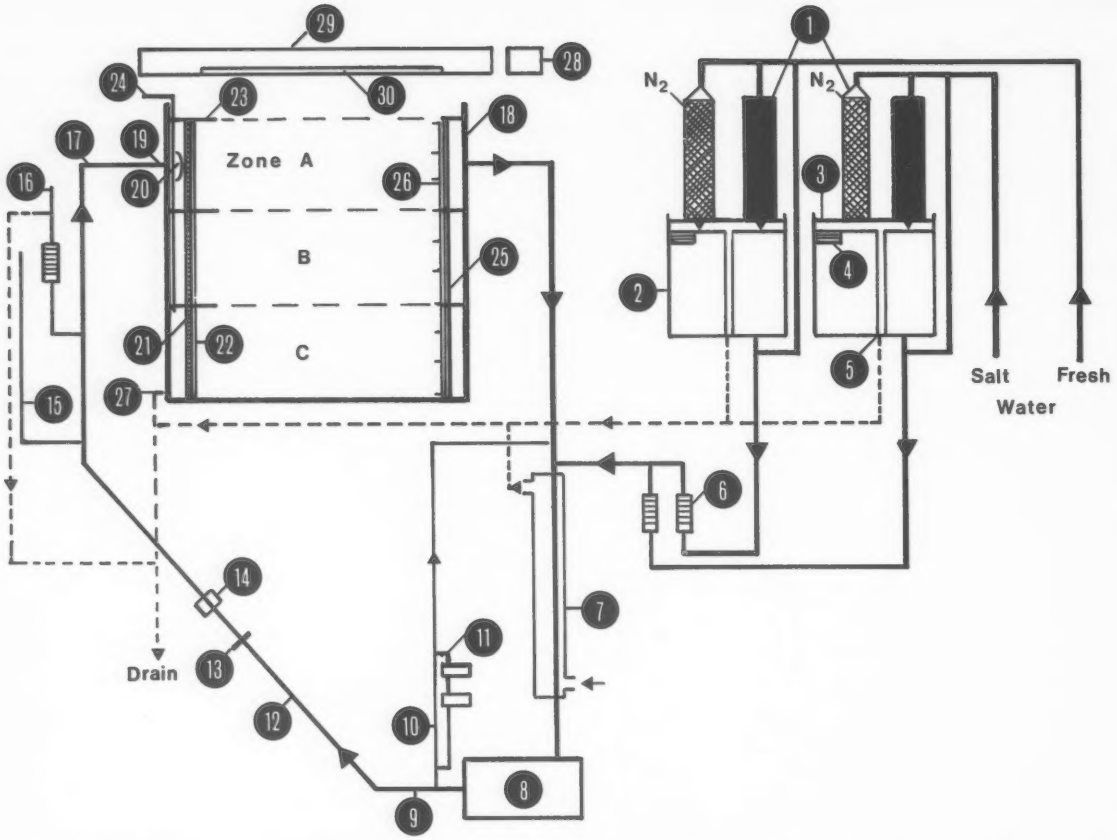

FIG. 1. Schematic showing the main components of the apparatus: 1, aeration–dearation columns; 2, constant head reservoirs; 3, perforated lid; 4, float switch; 5, drain; 6, water flow regulators; 7, heat exchanger; 8, water pump; 9, vertical pipe; 10, bypass–recirculation loop; 11, filters; 12, main water supply line to aquarium; 13, orifice plate; 14, main control valve; 15, vertical standpipe; 16, water level control standpipe; 17, sensor location; 18, aquarium (lateral view); 19, entry location of main water supply; 20, water dispersion cone; 21, water dispersion plate; 22, nylon screen, entry side; 23, acrylic horizontal wings; 24, capillary air bleeds; 25, nylon screen, exit side; 26, manifold system with sampling ports; 27, drain valve; 28, metal halide light source with photoperiod control; 29, Light Pipe™; 30, red fluorescent lights. Water circulation loops to the middle (B) and bottom (C) zones of the aquarium are omitted for clarity.

in that salinity, dissolved oxygen, water velocity, light intensity, and, to a lesser extent, temperature can be varied and controlled. Operation in the partial replacement or recirculation mode augments this flexibility. A detailed description of the apparatus is presented below and the main components are illustrated in Fig. 1.

Water Supply, Regulation, and Delivery

Freshwater is supplied from a well at the West Vancouver Laboratory at a temperature which varies seasonally from 10 to 11.5°C. Seawater is supplied directly from English Bay adjacent to the laboratory; the pump intake is situated approximately 100 m offshore at a depth of 15 m below the low tide level. Salinity and temperature vary seasonally from about 26 to 30‰ and from 7 to 12°C, respectively. The seawater is either cartridge filtered (30 μm) prior to admission or fed unfiltered into the system. Both freshwater and seawater are delivered through PVC pipes either directly to the apparatus or through deaeration or aeration columns (1) and 140-L polyethylene constant head reservoirs (2).

Aeration–deaeration is accomplished with insulated packed columns (Silver et al. 1963; Owsley 1981) 2.6 m × 10 cm diameter. Water entering the columns is fractured and dispersed by 2.5-cm "Flexi-rings" and enters the constant head reservoirs through a perforated acrylic lid (3) which is attached to the columns by a neoprene collar. Columns used for aeration purposes are open to the atmosphere, while those used for deaeration are enclosed with a neoprene collar through which butyl rubber tubing, attached to Micropore tubing, delivers nitrogen at a rate controlled by rotameters. Better deaeration was achieved when nitrogen was admitted to the top than to the bottom of the columns. The functions of both columns are interchangeable.

A float switch (4) coupled to a buzzer warning device is located in each reservoir; overflow water drains via a central standpipe (5). From the base of each reservoir, 5-cm-diameter PVC pipes convey water into the self-contained building

(located away from disturbance and vibration) which houses the apparatus. Here, three 2.5-cm-diameter lines branch from the main supply lines, one to each of the three water supply "loops" of the apparatus (for simplicity, the description will consider just one "loop"). Both freshwater and seawater flows are monitored with rotameters ($\leq$38 L·min^{-1}) and regulated with PVC ball valves. Separate freshwater and seawater lines join a common 5-cm pipe which then admits water to the main 10-cm-diameter line prior to the heat exchangers (7).

Double-walled aluminum heat exchangers are used to cool water circulating in the system. Municipal water is supplied to each heat exchanger, countercurrent to experimental water flows, through a 5-cm-diameter pipe. Since the degree of cooling is a product of the temperature differential and flow rate of cooling water (adjusted manually by means of gate valves), temperature adjustment is, at the present time, largely empirical and becomes more difficult as the temperature of municipal water rises in the summer. In 1985, this occurred most rapidly in June and July and an associated rise in total gas pressure due to heating within the system limited further experimentation at this time. We hope eventually to be able to provide precise temperature control with a closed-loop refrigeration system.

From the heat exchangers, experimental water is drawn into a 7.5-hp electrically powered centrifugal pump (8). Internal elements of the impellor and surfaces in contact with water are coated with epoxy to prevent contamination and corrosion.

Water exits the pump via a 7.5-cm-diameter vertical pipe (9) and flows either directly to the aquarium or is partially recirculated through a bypass loop (10) containing particle and activated charcoal filters (11), which may be isolated from the system as required, and back to the main water supply line upstream of the heat exchangers. A horizontal 15.0-cm-diameter pipe (12), which originates from the top of the vertical pipe from the pump, conveys water to the aquarium.

Water flow is monitored with a differential pressure flowmeter comprising a stainless steel orifice plate (13) and a readout gauge. Pressure differences have been calibrated against current velocities within the aquarium and operation of a butterfly valve (14) controls the flow of water into each zone.

Upstream of the aquarium, a vertical standpipe (15), which is open to the atmosphere, branches from the main supply line. It is used to introduce materials (e.g. dyes, effluents) or to withdraw samples for analysis. The standpipe also provides a port for the introduction of the dissolved oxygen sensor. Downstream, and closer to the aquarium, a 5-cm-diameter standpipe (16) connects the main water supply pipe to the overflow drain by flexible plastic tubing. This overflow standpipe, which is fitted with a rotameter, can be moved vertically by use of a worm gear and controls the water level of one zone in the aquarium. When operating in the continuous-flow mode, water inlet and exit flows for particular zones may be balanced and the volume in each zone adjusted, thereby controlling the relative separation or mixing of adjacent waters in the three zones A, B, and C.

Conductivity and temperature sensors are mounted in the main supply pipes to monitor waters just before they enter the aquarium (17). Manually operated air bleeds are located along the piping system at the top of the vertical pipe (9) from the pumps, along the bypass recirculation line (10), and on either side of the orifice plate (13). These are used during the initial stages of operation to avoid dissolved gas supersaturation in the apparatus.

Aquarium

The experimental chamber (18) is an acrylic tank (2.4 × 2.4 × 0.8 m; 19-mm wall thickness) which rests on a bed of sand to reduce vibration. Water enters (19) through a centrally positioned pipe into each of the three separately controlled but identical zones A, B, and C; all are approximately 76 cm in height.

Incoming water impinges upon a dispersion cone (20) attached to a perforated acrylic plate (21) mounted perpendicularly to the flow. The cone serves to reverse the direction of the bulk of the incoming water stream, distributing it within the small (8.5 cm wide) area between the tank wall and the plate. An asymmetrical pattern of 6-mm-diameter holes in the plate serves to even and distribute the flow horizontally as it enters the experimental chamber. Directly adjacent to the perforated plate is a nylon screen (1.0-mm mesh) mounted on an acrylic frame (22). This screen prevents organisms from entering the water inlet area and further assists in achieving laminar flow conditions.

Flow pattern tests utilizing dyes indicated a region of disturbed flow immediately downstream of partitions between adjacent zones. It was especially noticeable where adjacent water masses met and were mixed by turbulence. Once this was reduced by installing acrylic wings (23) horizontally across the top and bottom of zone A and between zones B and C, we obtained a sharp and stable halocline between zone A (freshwater) and zone B (seawater). A system of fine (1.4 mm ID) polyethylene capillary tubing extending to the areas beneath the wings, and behind the perforated plates, serves as air bleeds (24).

Prior to leaving the aquarium, water passes through a Nitex screen (25) identical to that which is located on the left, water-entry, side.

Depending upon experimental requirements, a manifold system (26) is installed on the right side of the tank to permit the abstraction of water samples at discrete depth intervals. The manifold comprises an acrylic strip fitted with 75-mm-long glass tubes (1.6-mm aperture) at fixed intervals along its length. Capillary tubing leads from the glass tubes to a remote-tapped syringe system through which water samples can be taken for analysis, without disturbing the experimental fish. A 5-cm-diameter ball-valve drain (27) is located on the bottom left side of the aquarium.

Lighting

A 400-W metal halide lamp (28) with spectral qualities similar to daylight provides a collimated light beam to an acrylic Light Pipe™ (TIR Systems Ltd.), 3.0 × 0.2 × 0.2 m (29), suspended horizontally and 0.3 m above the aquarium. Light energy (microEinsteins per second per square metre) is recorded using a Li-Cor LI 193SB spherical quantum sensor (system accuracy ±8%).

The Light Pipe™ transmits light horizontally by internal reflection and illuminates the tank from above. Although the degree of light penetration varies with turbidity, measurements in clear well water (Fig. 2) indicate that the attenuation of light intensity along the length of the Light Pipe™, which is apparent at the surface, is replaced at depth by a central peak which drops off longitudinally in a symmetrical fashion. An example of light distribution in the lateral plane at depth (38 cm) is shown in Fig. 3. These data indicate relatively uniform

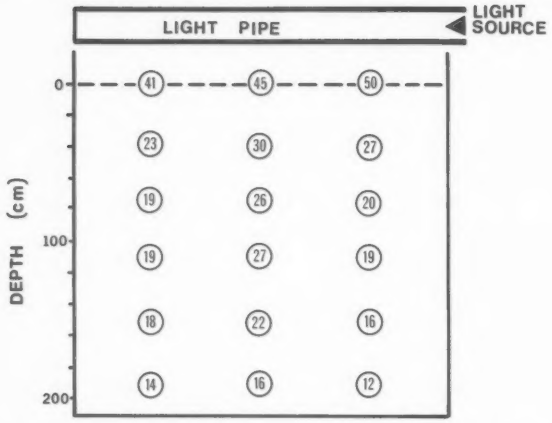

FIG. 2. Distribution of light intensities ($\mu E \cdot s^{-1} \cdot m^{-2}$) measured centrally along the length of the Light Pipe™ at various depths in clear well water.

illumination conditions, a pattern that persists with depth and represents an integration of light impinging on the spherical sensor from all directions, including that due to internal reflection within the aquarium. A black background is provided to the aquarium and a black PVC mat covers the bottom to reduce reflection.

Light energy levels similar to those which occur within the WCS were recorded under cloudy and rainy conditions in the estuary of the Campbell River, B.C., at 19:00 on May 30, 1984 (Piercey et al. 1985), where values ranged from 56 $\mu E \cdot s^{-1} \cdot m^{-2}$ at the surface to 24 $\mu E \cdot s^{-1} \cdot m^{-2}$ at 1.8 m depth. However, light energy readings within the aquarium are limited to approximately 2.5% of maximum values recorded in bright summer sunlight during the same study. In addition to removing all electrical components from the corrosive atmosphere immediately above the aquarium, the Light Pipe™ facilitates photoperiod control. An adjustable semicircular aluminum screen is rotated once every 24 h between the luminaire and the acrylic prism light guide by a precision-geared synchronous electrical motor. A 3.5-h dawn and dusk period is provided and seasonal adjustments are accomplished by varying the open sector of the adjustable shutter.

Red fluorescent lights (emission range 575–725 nm, peak 660 nm) provide illumination for filming at night and are kept on continuously. Two of these 1.2-m fluorescent tubes (30) are suspended on either side and along the length of the Light Pipe™.

Monitoring and Observation

A centrally located instrument panel houses meters which continuously monitor dissolved oxygen (milligrams per litre), temperature (degrees Celcius), and conductivity (microsiemens per centimetre), with an accuracy of ±1.5%, ±0.1°C, and ±2%, respectively. A multichannel chart recorder provides a permanent record of each variable at 3-min intervals.

Water samples collected from the manifolds (26) are analyzed for chloride amperometrically, with a Buchler–Cotlove Chloridometer™ adapted to operate on 10-μL samples, for osmolality (200 μL) with a Precision Systems Osmette S

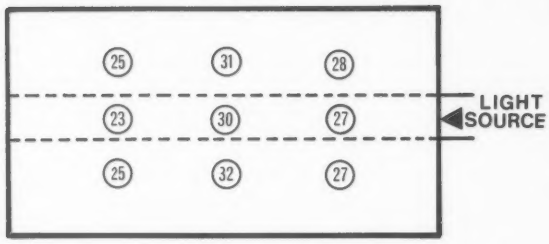

FIG. 3. Horizontal distribution of light intensity ($\mu E \cdot s^{-1} \cdot m^{-2}$) measured at a depth of 38 cm in clear well water.

Automatic Osmometer, and for dissolved oxygen (500 μL) using a Radiometer PHM 7lb Acid–Base Analyzer equipped with a Type E 5046 PO_2 electrode mounted in a thermostatted cell operated at the same temperature as the aquarium water.

Total dissolved gas pressure determinations are made before and after experiments using Novatech Designs Ltd. model 300 C tensionometers (accuracy ±0.3% within our operating range) and applying calculations provided by Colt (1984).

The velocity of water moving across each of the zones in the aquarium was determined by injecting rhodamine dye and following the changes in light energy with the quantum sensor coupled to a chart recorder. By operating the main control valve (14), flows were manipulated to generate calibration curves relating velocity to differential pressure gauge readings. Velocities within zones, at the same gauge settings, were similar and maximum velocities of 10–12 $cm \cdot s^{-1}$ were recorded. Under stratified conditions (freshwater above salt water), entrainment due to turbulence increases at the higher velocities but can be offset by the addition of more replacement water into adjacent zones.

Replacement times of water in the apparatus are changed by adjusting make-up water flows, and since the water volume of each loop in the system differs due to piping configurations, these vary very slightly among zones. The total volume of water in circulation is approximately 4900 L and, for example, with make-up water flows to each main loop (zone) of the apparatus of 18 or 36 $L \cdot min^{-1}$, 90% replacement would be approximately 3 and 1.75 h, respectively (Sprague 1969). Under normal continuous-flow operating conditions, water flows to each zone are fixed at levels between 19 and 26.5 $L \cdot min^{-1}$.

A high-resolution, closed-circuit black and white video system is used to record the behaviour of organisms in the aquarium. Extraneous light is completely occluded from the filming area by moveable curtains; similarly, light emanating from the Light Pipe™ is confined to the aquarium surface.

A high-resolution video camera (Panasonic WV-1850, 800 lines) fitted with an auto-iris lens (1:1.3, f = 12.5 mm) and an extended-red Newvicon™ tube facilitates observation over a broad range of lighting conditions. Since this tube has high sensitivity in the near infrared region (<940 nm), and a peak sensitivity at 780 nm, it is ideally suited to observation of nocturnal behaviour under low-level red light illumination.

The entire lateral side of the water column is observed directly on a high-resolution video monitor (850 lines) located outside the light-occluded area, or recorded for playback, albeit at reduced resolution (300 lines), on a time-lapse VHS recorder (Panasonic NV-8050). A single high-grade 120-mm videotape (12.7 mm) can record up to 240 h of observations; a time–date generator facilitates the precise timing of events.

TABLE 1. Physical conditions in the water column simulator during a continuous-flow stratified mode.

Zone[a]	Temperature (°C) $\bar{x} \pm$ SD	(*n*)	Salinity (‰) $\bar{x} \pm$ SD	(*n*)	Velocity ($cm \cdot s^{-1}$)	Replacement flow ($L \cdot min^{-1}$)	TGP (%)[b] $\bar{x} \pm$ SD	(*n*)
A	12.0±0.4	(140)	0.4±0.2	(140)	3–4	19 or 26.5	102.3±0.8	(10)
B	11.0±0.5	(140)	27.6±0.8	(140)	3–4	19 or 26.5	103.5±0.5	(16)
C	11.0±0.5	(140)	27.1±0.7	(140)	3–4	19 or 26.5	103.3±0.7	(16)

[a]Refer to Fig. 1.
[b]Total gas pressure, measured at the end of each experiment.

Although this system is suited to computerized image analysis techniques, at the present time we are using visual quantification of fish behaviour from tape recordings.

Vertical Stratification

Between May 9 and June 6, 1985, we conducted five experiments to observe behavioural responses of juvenile chinook salmon (*Oncorhynchus tshawytscha*) to reduced dissolved oxygen. Physical measurements collected during these experiments provide an example of the stability of the halocline and the maintenance of two discrete water masses under continuous-flow conditions.

During these experiments, the water column was stratified by continuous replacement of zone A with air-equilibrated well water and zones B and C with air-equilibrated seawater. Operating conditions are given in Table 1.

Once a stable halocline was achieved, dissolved oxygen levels in the freshwater zone were reduced by gradual replacement of saturated with deaerated water from a packed column operating at a 2:1 ratio of water to nitrogen. Admission of hypoxic water began at 07:30 and ended at 10:30 when air-equilibrated well water was readmitted. This protocol produced a continuously changing but controlled and reproducible variation in dissolved oxygen concentration. Fish behaviour was recorded in relation to this change.

Over the course of the experiments, water was continuously abstracted through nine ports on the manifold system (26, Fig. 1) spaced at 20-mm intervals across the interface between zones A and B, at the exit side of the aquarium. Variations in dissolved oxygen levels in water sampled from discrete depths by the manifold ports are presented in Fig. 4. For simplicity, only data from ports 1, 5, 6, and 8 have been used to reveal the changes in dissolved oxygen across the halocline. Between ports 5 and 6, a vertical distance of 20 mm, the sharp gradient in PO_2 indicates a clear separation of the two water masses and little diffusive exchange during the course of these experiments. There was, however, a slight depression of dissolved oxygen levels in water samples taken through port 6 ($\bar{x} \pm$ SD = 145 ± 10 Torr, n = 70) relative to those from port 8 (153 ± 4 Torr, n = 70). Corresponding average salinities (n = 35) were 0.6 ± 0.4, 2.5 ± 2.6, 26.0 ± 3.0, and 28.8 ± 1.4‰ for waters taken from ports 1, 5, 6, and 8, respectively, confirming the high degree of vertical stratification and the stable nature of the water column under continuous-flow conditions.

Application to Fish Behaviour Research

Underyearling juvenile chinook salmon (Chehalis Hatchery, Harrison River stock, Fraser River system) reared in air-equilibrated well water at the West Vancouver Laboratory from March 1985 were used in experiments that examined their seasonal behavioural response to seawater under simulated vertically stratified estuarine conditions and to reductions in dissolved oxygen in the freshwater layer. Although the results of these experiments will be reported elsewhere, we provide information on the vertical distribution of salmon in relation to changes in dissolved oxygen to illustrate the nature of our work and the conditions which may be controlled in the apparatus.

The chinook salmon used in the experiments had been held in 6850-L circular fibreglass tanks, 3 m diameter, in 0.65 m depth of water. Special precautions were followed during rearing to minimize disturbance, and automatic feeders continuously presented food (Oregon Moist Pellet) at approximately 2% body weight$\cdot d^{-1}$ from dawn to dusk. This was done to avoid behavioural entrainment on the customary twice-daily feeding schedule at our aquarium facility.

Fish were anaesthetized lightly (approximately 30 s at 170 mg MS-222$\cdot L^{-1}$; Sandoz) to help in counting and then transferred into 50-L nylon mesh baskets suspended in the holding tanks, where they were starved for 24 h. Subsequent transfer to the WCS was accomplished by placing the baskets in a submerged container and gently immersing and releasing the contents into the experimental aquarium, without further exposure to the air.

The fish ($\bar{x} \pm$ SD = 2.95 ± 0.79 g, 67.7 ± 6.1 mm, n = 20, May 24, 1985) were left for 48 h (freshwater only) to recover from transfer and acclimate to new conditions in the WCS. The aquarium was then stratified with freshwater (zone A) overlying two zones (B and C) of seawater. After 18 h, hypoxic water was introduced at 07:30 to zone A. At 10:30, air-equilibrated freshwater was once more reintroduced into the system, creating a somewhat asymmetric "oxygen sag curve."

During the experiment, dissolved oxygen, salinity, and temperature were monitored. These recordings are summarized in Table 2 and reveal the stability of conditions within the three horizontally flowing zones and the range of dissolved oxygen levels in the freshwater layer.

Although the distribution of salmon was recorded continuously, the nature of their response to variation in dissolved oxygen in the freshwater zone A is illustrated by histograms at precise 0.5-h intervals (Fig. 5). While fish moved between zones before the lowering of dissolved oxygen levels, they tended to utilize the freshwater zone most frequently. Depression of dissolved oxygen resulted in a change in this behaviour. The fish moved downwards in the water column, some aggregating at the interface (halocline) between the freshwater of zone A and the seawater zones B and C (e.g.

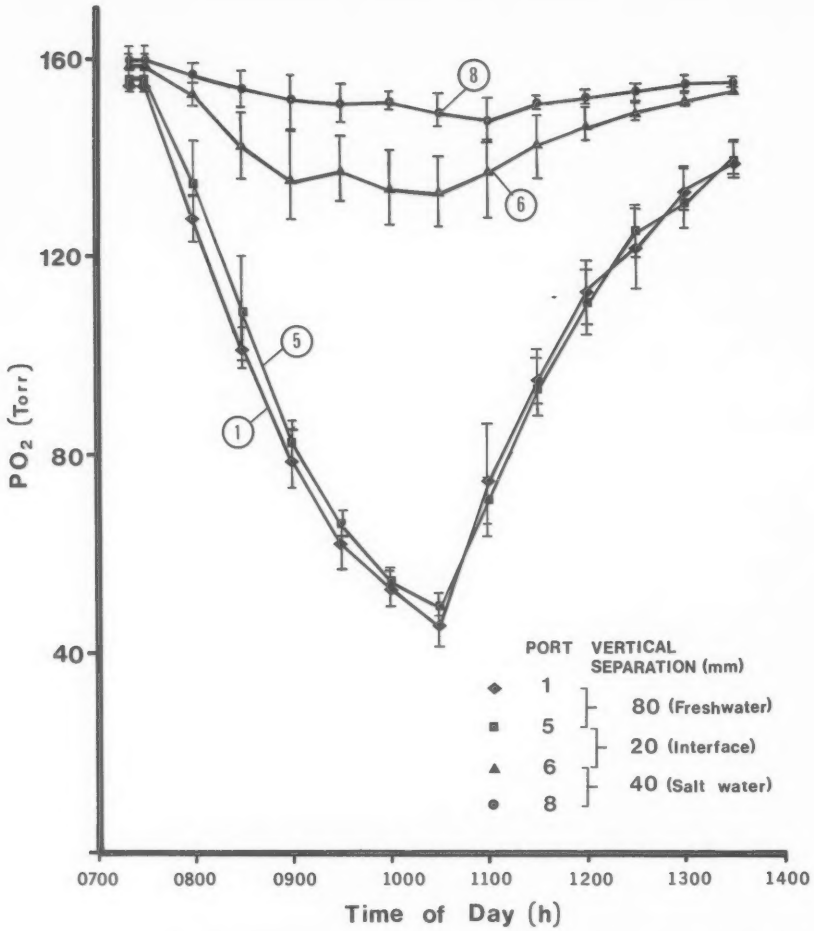

FIG. 4. Variation in the partial pressure of dissolved oxygen across the halocline in a vertically stratified water column during five experiments in which dissolved oxygen was manipulated in the freshwater layer. Data points represent mean values ± SD. For sampling port location, refer to Fig. 1.

TABLE 2. Mean values (± SD, n = 68) of dissolved oxygen, salinity, and temperature recorded in water (3–4 cm·s^{-1}) in zones A, B, and C, May 24, 1985.

Variable	Zone		
	Top (A)	Middle (B)	Bottom (C)
Dissolved oxygen (mg·L^{-1})	2.7–10.5[a]	9.4±0.1	9.3±0.1
Salinity (‰)	0.7±0.3	27.8±0.2	27.5±0.3
Temperature (°C)	12.2±0.2	11.4±0.2	11.5±0.2

[a]Range.

08:30, Fig. 5). Prolonged avoidance of the hypoxic water was observed but the fish made transient excursions into this freshwater zone. Upon raising the dissolved oxygen levels in zone A, the fish again tended to occupy freshwater rather than the deeper seawater zones. This experiment was repeated over a number of months to examine temporal variation in the behavioural response of these fish to the experimental conditions.

Improvements and Limitations

Although a number of improvements to the apparatus are desirable, primarily associated with greater temperature control, nighttime illumination, and image processing, we have demonstrated thus far that the WCS can provide a reproducible simple two-layer vertical stratification. Eventually we hope to

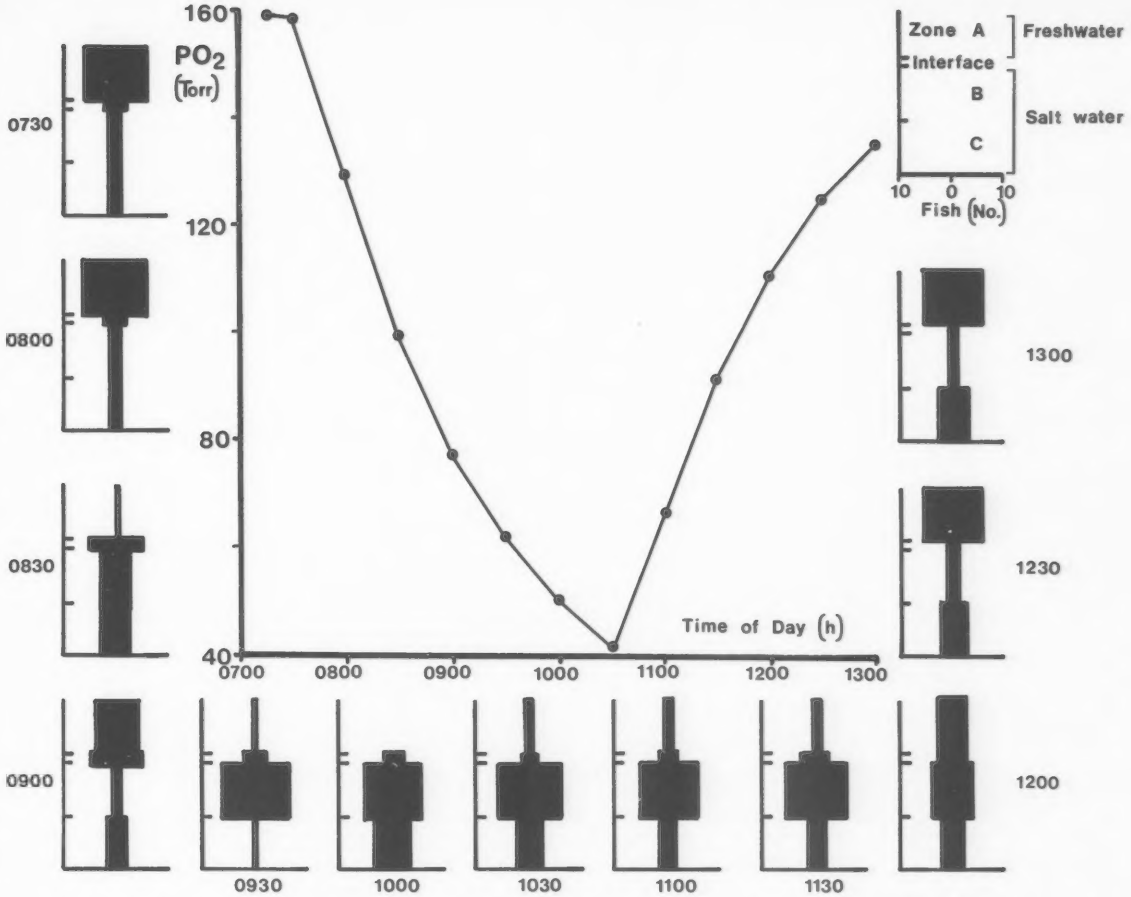

FIG. 5. Variation in the partial pressure of dissolved oxygen in freshwater overlying seawater in a vertically stratified water column, May 24, 1985. Histograms represent the distribution of 20 fish within the water column at 0.5-h intervals.

provide a more gradual transition from freshwater to seawater through intermediate salinities in the middle zone.

Experiments on the effects of increased water temperatures on fish behaviour are limited by gas supersaturation in the system. This potential problem could be overcome by passing tempered water through packed columns before admission to the aquarium. Although we have managed to maintain total gas pressure (TGP) in the system at values less than 103–104%, the possible influence of variations in O_2:N_2 ratios at low (102–103%) TGP levels is unknown. We are unable to find information on behavioural changes at these low levels of TGP.

At the present time, "night" illumination consists of red fluorescent fixtures mounted on the outside and along the length of the Light Pipe™. These were installed to take advantage of the high sensitivity of our video camera in the far-red region of the light spectrum where, at the same time, the sensitivity of fish vision is thought to be greatly reduced, especially for the dark-adapted retina. Since our fluorescent lights peak at 660 nm, near the upper limits of the region of relatively high spectral sensitivity in light-adapted teleost retinae (Protasov 1968) and our light meter sensitivity is limited to <700 nm, we are unable to quantify the actual light energy available to the fish. Thus we cannot determine whether our fish are light or dark adapted under our present "night" conditions and, therefore, whether the downward shift in spectral sensitivity accompanying scotopic vision (Purkinje shift) has in fact occurred. Preliminary experiments do suggest, however, that the vertical distribution of juvenile coho salmon (*O. kisutch*) differs between day and night in our stratified water column.

We are presently investigating the use of filters to modify the spectrum of the incident light from the Light Pipe™ luminaire. Since any modification of the collimated light beam will be transmitted through the light prism, this system should facilitate the study of behaviour of organisms in relation to controlled manipulation of light spectrum, photoperiod, and intensity. A more detailed discussion of the application of the Light Pipe™ for behavioural work is the subject of a subsequent publication.

Long-term plans for the WCS include monitoring the behavioural changes in juvenile chinook salmon subjected to (a) preexposure to contaminants or mixed effluents, (b) addition of contaminants or mixed effluents to specific zones in the

aquarium — a stratified continuous-flow sublethal behavioural bioassay, (c) predator–prey interactions, i.e. subtle changes in behaviour due to pollutants which might render organisms more vulnerable to predation (Goodyear 1972; Kania and O'Hara 1974), and (d) responses to a, b, and c under hypoxia. The WCS at its current stage of development provides a tool to study interactions of salinity, dissolved oxygen, and pollutants on the behaviour of juvenile salmon and other organisms.

Acknowledgments

Western Canada Hydraulics Ltd. assisted in the design and construction of the apparatus. H. Herunter, R. Lauzier, C. McPherson, G. E. Piercey, S. Spohn, and other staff at the West Vancouver Laboratory provided invaluable assistance during development and experimental phases. L. Vetter and D. Price typed the manuscript.

References

BEAK CONSULTANTS LTD. 1981. Environmental impact statement of proposed improvements to the Fraser River shipping channel. Public Works Canada, Vancouver, B.C.

BIRTWELL, I. K. 1977. A field technique for studying the avoidance of fish to pollutants. Environ. Protect. Serv. Tech. Rep. No. EPS-5-AR-77-1, Halifax, N.S.: 69–86.

BIRTWELL, I. K., AND R. M. HARBO. 1980. Pulp mill impact studies at Port Mellon and Port Alberni, B.C. Trans. Tech. Sect. Can. Pulp Pap. Assoc. 81(12): 85–89.

BIRTWELL, I. K., S. NELLES, AND R. M. HARBO. 1983. A brief investigation of fish in the surface waters of the Somass estuary, Port Alberni, British Columbia. Can. MS Rep. Fish. Aquat. Sci. 1744: 31 p.

BRAWN, V. M. 1982. Behaviour of Atlantic salmon (*Salmo salar*) during suspended migration in an estuary, Sheet Harbour, Nova Scotia, observed visually and by ultrasonic tracking. Can. J. Fish. Aquat. Sci. 39: 248–256.

BRETT, J. R. 1952. Temperature tolerance in young Pacific salmon, genus *Oncorhynchus*. J. Fish. Res. Board Can. 9: 265–323.

COLT, J. 1984. Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. Spec. Publ. No. 14. American Fisheries Society, Washington, DC. 154 p.

GIATTINA, J. D., AND R. R. GARTON. 1983. A review of the preference–avoidance responses of fishes to aquatic contaminants. Residue Rev. 87: 43–90.

GOODYEAR, C. P. 1972. A simple technique for detecting effects of toxicants or other stresses on a predator–prey interaction. Trans. Am. Fish. Soc. 101 (2): 367–370.

GORDON, D. K., AND C. D. LEVINGS. 1984. Seasonal changes of inshore fish populations on Sturgeon and Roberts Banks, Fraser River estuary, British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1240: 81 p.

HEALEY, M. C. 1979. Detritus and juvenile salmon production in the Nanaimo estuary: 1. Production and feeding rates of juvenile chum salmon (*Oncorhynchus keta*). J. Fish. Res. Board Can. 36: 488–496.

1980. Utilization of the Nanaimo River estuary by juvenile chinook salmon, *Oncorhynchus tshawytscha*. Fish. Bull. 77: 653–668.

1982. Juvenile Pacific salmon in estuaries: the life support system, p. 315–341. *In* V. W. Kennedy [ed.] Estuarine comparisons. Academic Press, London, England.

HURLEY, D. A., AND W. L. WOODALL. 1968. Responses of young pink salmon to vertical temperature and salinity gradients. Int. Pac. Salmon Fish. Comm. Prog. Rep. 19: 1–80.

IWATA, M., AND S. KOMATSU. 1984. Importance of estuarine residence for adaptation of chum salmon (*Oncorhynchus keta*) fry to seawater. Can. J. Fish. Aquat. Sci. 41: 744–749.

KANIA, H. J., AND J. O'HARA. 1974. Behavioural alterations in a simple predator–prey system due to sublethal exposure to mercury. Trans. Am. Fish. Soc. 103(1): 134–136.

LARRICK, S. R., K. L. DICKSON, D. S. CHERRY, AND J. CAIRNS JR. 1978. Determining fish avoidance of polluted water. Hydrobiologia: 61(3): 257–265.

LEVINGS, C. D., G. L. GREER, AND P. MILLER. 1983. Results of mark–recapture experiments with juvenile salmonids on Sturgeon and Roberts Bank, Fraser River estuary. Can. MS Rep. Fish. Aquat. Sci. 1684: 27 p.

LEVY, D. A., AND T. G. NORTHCOTE. 1981. The distribution and abundance of juvenile salmon in marsh habitats of the Fraser River estuary. Univ. B.C. Westwater Res. Cent. Tech. Rep. 25: 117 p.

1982. Juvenile salmon residency in a marsh area of the Fraser River estuary. Can. J. Fish. Aquat. Sci. 39: 270–276.

MACDONALD, A. L. 1984. Seasonal use of nearshore intertidal habitats by juvenile Pacific salmon on the delta front of the Fraser River estuary, British Columbia. M.Sc. thesis, University of Victoria, Victoria, B.C. 187 p.

MACDONALD, J. S., I. K. BIRTWELL, AND G. M. KRUZYNSKI. 1987. Food and habitat utilization by juvenile salmonids in the Campbell River estuary. Can. J. Fish. Aquat. Sci. 44: 1233–1246.

MACDONALD, J. S., G. M. KRUZYNSKI, I. K. BIRTWELL, K. D. SEAMAN, L. DELMISTRO, AND M. KOTYK. 1985. Juvenile salmonid food and plankton samples from an inner and an outer estuarine station in the Campbell River estuary, British Columbia, 1984. Can. Data Rep. Fish. Aquat. Sci. 520: 27 p.

MACE, P. M. 1983. Bird predation on juvenile salmonids in the Big Qualicum River, Vancouver Island. Can. Tech. Rep. Fish. Aquat. Sci. 1117: 79 p.

MASON, J. C. 1974. Behavioural ecology of chum salmon fry (*Oncorhynchus keta*) in a small estuary. J. Fish. Res. Board Can. 31: 83–92.

MCCAULEY, R. W. 1977. Laboratory methods for determining temperature preference. J. Fish. Res. Board Can. 34: 749–752.

MCGREER, E. R., AND G. A. VIGERS. 1983. Development and validation of an in-situ fish preference–avoidance technique for environmental monitoring of pulp mill effluents, p. 519–529. *In* W. E. Bishop, R. D. Cardwell, and B. B. Heidolph [ed.] Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP802. American Society for Testing and Materials, Philadelphia, PA.

MCINERNEY, J. E. 1964. Salinity preference: an orientation mechanism in salmon migration. J. Fish. Res. Board Can. 21: 995–1017.

OLLA, B. L., W. W. MARCHIONI, AND H. M. KATZ. 1967. A large experimental aquarium system for marine pelagic fishes. Trans. Am. Fish. Soc. 96(2): 143–150.

OWSLEY, D. E. 1981. Nitrogen gas removal using packed columns, p. 71–82. *In* L. J. Allen and E. C. Kinney [ed.] Proceedings of the Bio-Engineering Symposium for Fish Culture. Fish Culture Section of the American Fisheries Society. FCS Publ. 1.

PIERCEY, G. E., I. K. BIRTWELL, H. HERUNTER, M. KOTYK, G. M. KRUZYNSKI, J. S. MACDONALD, AND K. SEAMAN. 1985. Data report on physical habitat characteristics and observations of fish at two regions in the estuary of the Campbell River, British Columbia, 1984. Can. Data Rep. Fish. Aquat. Sci. 551: 107 p.

PROTASOV, V. R. 1968. Vision and near orientation of fish. Academy of Sciences of the USSR. (Translated 1970, Israel Program for Scientific Translations, Jerusalem, Israel)

PURCELL, L. J., AND J. B. SHRODE. 1983. Vertical temperature preference tank for larval and juvenile fish. Prog. Fish Cult. 45(1): 27–29.

RAND, G. M. 1985. Behaviour, p. 221–263. *In* G. M. Rand and S. R. Petrocelli [ed.] Fundamentals of aquatic toxicology. Hemisphere Publishing Corporation, New York, NY.

REIMERS, P. E. 1973. The length of residence of juvenile fall chinook salmon in Sixes River, Oregon. Oreg. Fish. Comm. Res. Rep. 4(2): 43 p.

SCHERER, E. 1976. Overhead-light intensity and vertical positioning of the walleye, *Stizostedion vitreum vitreum*. J. Fish. Res. Board Can. 33: 289–292.

SILVER, S. J., C. E. WARREN, AND P. DOUDOROFF. 1963. Dissolved oxygen requirements of developing steelhead trout and chinook salmon embryos at different water velocities. Trans. Am. Fish. Soc. 92(4): 327–343.

SPRAGUE, J. B. 1969. Review paper: measurement of pollutant toxicity to fish: 1. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

WESTERBERG, H. 1982. Monitoring fish behaviour in relation to hydrographic fine structure, p. 154–166. *In* D. G. Pincock [ed.] Proceedings of the 4th International Conference on Wildlife Biotelemetry, Halifax, N.S.

1984. The orientation of fish and the vertical stratification at fine and micro-structure levels, p. 179–204. *In* J. D. Cleave, W. H. Neill, J. J. Dodson, and G. P. Arnold [ed.] Mechanisms of migration in fishes. Plenum Publishing Corp., New York, NY.

Plasma Testosterone Concentration Predicts the Ovulatory Response of Coho Salmon (*Oncorhynchus kisutch*) to Gonadotropin-Releasing Hormone Analog[1]

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Plasma concentrations of testosterone, 17α-hydroxy-20β-dihydroprogesterone (DHP), and estradiol-17β were determined in hatchery populations of adult female coho salmon (*Oncorhynchus kisutch*) before and after the fish were injected with gonadotropin-releasing hormone analog (GnRHa) to accelerate ovulation. Treatment with GnRHa significantly accelerated maturation over that observed in saline-injected controls; however, a number of females that received GnRHa did not ovulate for as long as 8 wk after treatment. Before treatment, the mean plasma levels of DHP did not differ between early- and late-ovulating females; estradiol differed sometimes, but not always; and testosterone was always higher in the early-ovulating fish. Thus, plasma concentrations of testosterone appear to be useful for predicting the sensitivity of coho salmon to GnRHa treatment for induced ovulation.

Les auteurs ont déterminé les concentrations plasmatiques de testostérone, de 17α-hydroxy-20β-dihydroprogestérone (DHP) et de l'estradiol-17β chez des populations de saumon coho (*Oncorhynchus kisutch*) femelle adulte d'élevage avant et après l'injection d'un analogue de l'hormone libératrice de la gonadotrophine (GnRHa) pour accélérer l'ovulation. Le traitement au GnRHa a nettement accéléré la maturation par rapport aux femelles témoins auxquelles on avait injecté une solution saline; toutefois, un certain nombre de femelles auxquelles on a injecté du GnRHa ont pris jusqu'à huit semaines pour ovuler après le traitement. Avant celui-ci, les teneurs moyennes en DHP plasmatique n'étaient pas différentes chez les femelles à ovulation précoce ou tardive; toutefois, les teneurs en estradiol variaient parfois et les teneurs en testostérone étaient toujours plus élevées chez les poissons à ovulation précoce. Il semble donc que les concentrations plasmatiques de testostérone peuvent servir à prédire la sensibilité du saumon coho au traitement par GnRHa pour induire l'ovulation.

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Final ovarian maturation in salmonid fishes, which is regulated by the endocrine system (Goetz 1983), is accompanied by dynamic changes in the plasma levels of steriods (Scott et al. 1983) and gonadotropin (Van Der Kraak et al. 1983). Specifically, circulating levels of both gonadotropin and 17α,20β-dihydroxy-4-pregnen-3-one (17α-hydroxy-20β-dihydroprogesterone or DHP) rise and the level of estradiol-17β decreases (Van Der Kraak et al. 1983, 1985). Gonadotropin, secreted by the pituitary, induces the release of DHP in the ovary, and DHP, in turn, stimulates the final maturation of oocytes (Jalabert 1976).

A gonadotropin-releasing hormone (GnRH) has been isolated and characterized from the hypothalamus of chum salmon, *Oncorhynchus keta* (Sherwood et al. 1983). Synthetic analogs of GnRH (GnRHa) have been used successfully to accelerate final maturation in coho salmon, *O. kisutch* (Van Der Kraak et al. 1983, 1985; Fitzpatrick et al. 1984), Atlantic salmon, *Salmo salar* (Crim et al. 1983a; Crim and Glebe 1984), and rainbow trout, *S. gairdneri* (Crim et al. 1983b). Treatment of female coho salmon with GnRHa elevated plasma gonadotropin (Van Der Kraak et al. 1983) and DHP (Van Der Kraak et al. 1984). In female coho salmon and steelhead trout (*S. gairdneri*), plasma estradiol was depressed and plasma androgens were elevated after treatment with serial injections of salmon gonadotropin and GnRHa (Sower et al. 1984). These studies demonstrated that specific changes occur in the endocrine system after treatment with maturation-inducing hor-

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TABLE 1. Experimental design (b = blood sampled; i = GnRHa or saline injected; c = ovulation checked).

1984					
n	T_0 16 Oct.	19 Oct.	T_0' 23 Oct.	26 Oct.	30 Oct. – 11 Dec.
50 mature	b, i	i	c	c	c
50 less-mature	b, i	i	c	c	c
50 mature			b, i	i	c
50 less-mature			b, i	i	c

1985				
n	T_{-7} 15 Oct.	T_0 22 Oct.	T_2 24 Oct.	31 Oct. – 3 Dec.
66	b	b, i	b, i	c

mones such as GnRHa. Emphasis has usually been placed on subsets of fish within the experimental populations that ovulate soon after treatment.

Less well studied are the fish that do not respond quickly to treatment. A large percentage of female Atlantic salmon did not ovulate when given GnRHa 45 d before the mean spawning date of control fish. Furthermore, these nonresponsive fish did not show the characteristic elevation of plasma gonadotropin seen in responsive fish (Crim and Glebe 1984). Similarly, in coho salmon, plasma steroid profiles in females that did not ovulate within 2 wk after GnRHa injection differed from those in females that ovulated within 2 wk (Van Der Kraak et al. 1985). Thus, status of maturation, as reflected by the endocrine system, may determine the sensitivity of the female to GnRHa.

Plasma concentrations of estradiol, DHP, and testosterone are highly correlated with the developmental stages of the ovaries of coho salmon during final maturation (Fitzpatrick et al. 1986). The objective of the present study, conducted at the freshwater hatchery of Oregon Aqua Foods, Inc., Springfield, OR, during fall 1984 and fall 1985, was to determine if the plasma concentrations of certain steroids could be used to predict the ovulatory response of coho salmon to GnRHa.

Materials and Methods

Experimental Animals

On 16 October 1984 (T_0), 200 females were sorted from the hatchery brood stock. Of these, 100 were from a population designated "mature," which had been at the hatchery longer than any other group of fish (having returned earlier from the ocean). The coloration of all members of this group indicated advanced sexual maturity. The other 100 fish were from a group designated "less-mature," which had been at the hatchery for the shortest time. Coloration of members of this group differed from that of fish of the "mature" group. On T_0, 50 "mature" and 50 "less-mature" fish were tagged, sampled, and treated. On 23 October 1984 (T_0'), the remaining untreated females (50 each) from the original "mature" and "less-mature" groups were tagged, sampled, and treated.

Eleven females from the "mature" group died during anesthesia on 23 October, and to make up for this loss, 20 females from the original raceway that contained the "mature" group of fish were then injected on 24 October. As controls for the injection process, 26 uninjected females from the original "mature" group raceway were placed with the experimental animals.

On 15 October 1985 (T_{-7}), 66 females were isolated from the hatchery brood stock; 44 were selected from a group of fish that had returned earlier than any other group and were therefore expected to mature before those of any other group, and the remaining 22 females came from another raceway containing animals that were not expected to mature as soon as the first group.

Sampling and Treatment

In 1984, we sampled blood from each female on the day of first injection with either GnRHa (des-Gly^{10}[D-Ala^6]LH-RH-ethylamide; Syndel Biochemicals) or saline (T_0 or T_0') (see Table 1). The females received a second injection of either GnRHa or saline 3 d later. In 1985, we sampled blood from each female 1 wk before first injection with GnRHa or saline (T_{-7}), on the day of injection (T_0), and on the day of second injection 2 d later (T_2).

We anesthetized females with ethyl *p*-aminobenzoate (benzocaine), tagged them individually, and collected a blood sample from the caudal vein with a Vacutainer (Becton–Dickinson) coated with lithium heparin. On the treatment days, the fish were then injected intraperitoneally either with 5 μg of GnRHa (about 2.0 μg/kg body weight) or with a saline solution, as described by Fitzpatrick et al. (1984). All fish were checked manually for ovulation every 3–4 d in 1984 and 4–6 d in 1985 for the first month after injection and every week thereafter. Females were considered to have ovulated if eggs were extruded when gentle pressure was applied to the abdomen. Ovulated fish were removed from the experimental population. In 1984, the experiment lasted until all the females had ovulated (48 and 49 d after T_0 and T_0', respectively). In 1985, females that ovulated on day 45 or thereafter (designated 45+) were grouped together for analysis.

In 1984, a total of 18 females died before ovulation, but they were still used to calculate the cumulative percentages of fish that had ovulated in each treatment group. Nine other fish divided among four different treatment groups, which were mistakenly killed before complete ovulation, were not considered in the analysis because it was impossible to determine the date of ovulation. Mortality was equal in all treatment groups.

In 1985, a total of four fish either died during the course of the experiment or lost their tags and were not considered in the analysis except when we determined the cumulative percentage of ovulation in the treatment group. Two fish killed before complete ovulation were not considered in the analysis. When a single fish constituted the total number of females ovulating on a particular date, it was not considered in the statistical analysis of plasma steroid content. Since we were interested in the value of circulating steroids in predicting the ovulatory response after injection of GnRHa, we combined data on steroid levels from all GnRHa-treated animals for statistical analyses. Three females treated with GnRHa in 1985 had high levels of DHP on the day of first injection, suggesting that they were very close to ovulation before treatment (all ovulated by day 9); these fish were not used in the statistical analysis of plasma steroid content.

Assays

The blood samples were held on ice until centrifugation, after which the plasma was drawn off and stored frozen (−20°C) until analysis. Plasma samples were analyzed for DHP, estradiol-17β, and testosterone by radioimmunoassay using the procedure of Fitzpatrick et al. (1986) with the exception that a single aliquot of 50 μL of plasma was extracted with diethyl ether, the extract was reconstituted in 1 mL of phosphate buffered saline, and then portions of reconstituted extract were assayed for each steroid.

Statistics

Data for time-elapsed from the first injection to ovulation between GnRHa- and saline-treated groups were analyzed by a Mann−Whitney U test. The plasma steroid data for individuals through the three sampling dates in 1985 were analyzed initially by Friedman's analysis of variance for dependent samples. Data showing significant variation were then subjected to Wilcoxon's sign test to determine significant differences between the sampling dates. Groups were established based on the number of days elapsed to ovulation; steroid data were then compared between groups for any particular sampling date and treatment by Kruskal−Wallis analysis of variance for independent samples. Groups demonstrating significant variation were then analyzed with a Mann−Whitney U test. Definitive statistical significance was set at $p < 0.05$.

Results

Inducement of Ovulation

Fish of the "mature" group that received GnRHa on 16 October 1984 (T_0) ovulated in significantly fewer days than did saline-injected controls, but this effect was not apparent in the "less mature" groups (Fig. 1). Fish treated with GnRHa on 23 October 1984 (T_0') from both the "mature" and "less-mature" groups ovulated sooner than their respective saline-injected controls; likewise, "mature" fish injected with saline on T_0' ovulated sooner than uninjected "mature" controls.

In 1985, females that received GnRHa ovulated in significantly fewer days than did saline-injected control fish (Fig. 1): 21.1 ± 2.4 vs. 36.1 ± 3.3 (mean ± SE).

Analysis of Steroid Concentrations

Plasma concentrations of hormones on T_0 and T_0' in 1984 were analyzed as a function of the number of days elapsed to ovulation after treatment. Data were combined for fish that received the same treatment on the same date regardless of maturity status. The 1985 data for the plasma steroid levels were analyzed in two ways, in both of which the fish were grouped by the number of days elapsed to ovulation. The first analysis compared values temporally in fish from any particular group (ovulating 9, 14, or 45+ d after treatment) between the three sampling dates (T_{-7}, T_0, and T_2); the second compared the values between groups on a given sampling date. For example, first the levels of DHP in all females that ovulated 9 d after GnRHa injection were compared between the various sampling dates (T_{-7}, T_0, and T_2); second, DHP in females that ovulated after 9 d was compared with that of females that ovulated after 14 and 45+ d on any given sampling date (T_{-7}, T_0, and T_2).

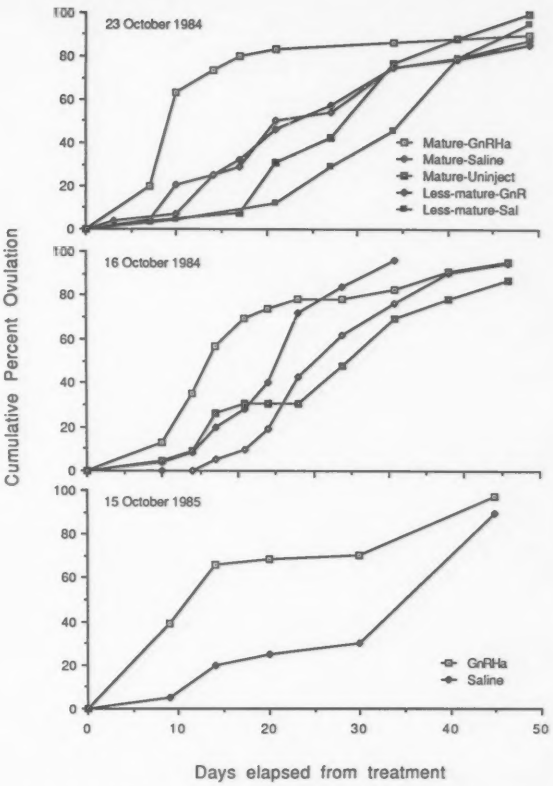


FIG. 1. Induced ovulation of coho salmon treated on 16 and 23 October 1984 and on 15 October 1985. The numbers of fish that ovulated after being injected with GnRHa or saline, or were not injected, are expressed as cumulative percentages of their respective groups.

The numbers of control females that ovulated on either 9, 14, or 30 d after saline injection were too small to allow a rigorous statistical comparison of plasma hormone levels over the sampling period; nonetheless, trends were apparent (Table 2) as described below.

17α,20β-Dihydroxy-4-pregnen-3-one

In 1984, the levels of DHP on T_0 or T_0' did not vary as a function of days elapsed to ovulation after treatment with either GnRHa or saline (Fig. 2).

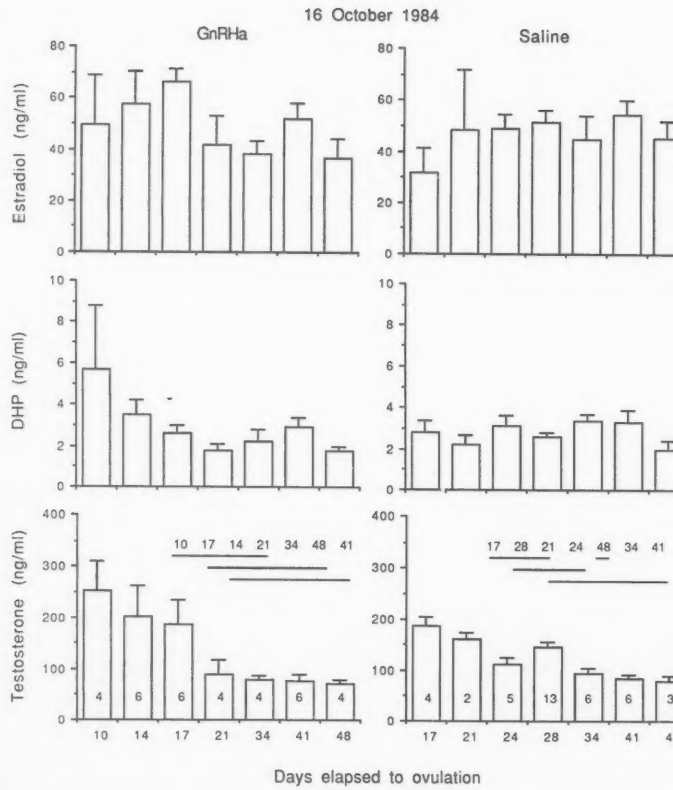
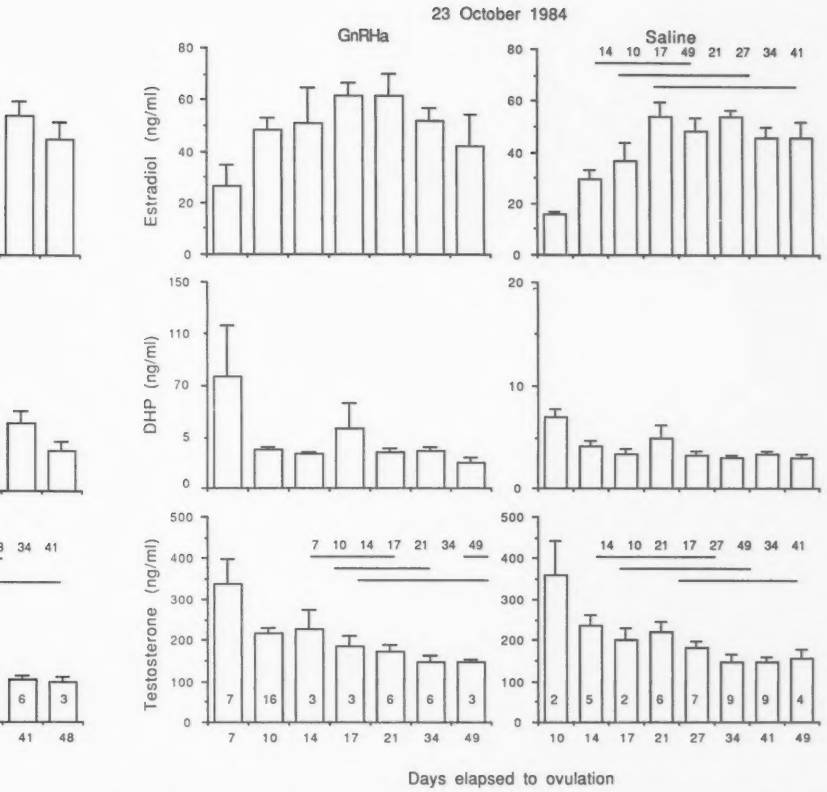

16 October 1984
GnRHa
Saline
Estradiol (ng/ml)
DHP (ng/ml)
Testosterone (ng/ml)
10 17 14 21 34 48 41
17 28 21 24 48 34 41
4 6 6 4 4 6 4
4 2 5 13 6 6
10 14 17 21 34 41 48
17 21 24 28 34 41
Days elapsed to ovulation

FIG. 2. Mean plasma concentrations of estradiol-17β, 17α,20β-dihydroxy-4-pregnen-3- gonadotropin-releasing hormone analog (GnRHa) or saline. Each bar and vertical line re by the number of days elapsed to ovulation from the date of treatment (16 or 23 October 1 significantly different (note: minimum sample size matrices required by the Mann-Whitne



regnen-3-one (DHP), and testosterone in female coho salmon on the day of treatment with either
al line represents the mean and standard error for the indicated number of females in groups differentiated
October 1984). Means of groups identified by days to ovulation which share the same horizontal line are not
n-Whitney test are 2 × 8, 3 × 5, or 4 × 4).

TABLE 2. Plasma levels of steroids (ng/mL) in female coho salmon injected with saline on both 22 and 24 October 1985. Days to ovulation indicate the time elapsed from 22 October to ovulation.

Days to ovulation	n	17α,20β-Dihydroxy-4-pregnen-3-one			Estradiol-17β			Testosterone		
		T_{-7}	T_0	T_2	T_{-7}	T_0	T_2	T_{-7}	T_0	T_2
9	1	4	350	685	59	4	3	486	736	448
14	3	3 ± 0.4	7 ± 0.6	13 ± 6	54 ± 9	28 ± 12	19 ± 12	398 ± 76	442 ± 76	545 ± 131
30	2	2 ± 0.5	3 ± 0.3	4 ± 0.5	77 ± 26	50 ± 14	38 ± 18	261 ± 12	329 ± 40	340 ± 52
45+	12	2 ± 0.1	3 ± 0.2	3 ± 0.2	70 ± 3	60 ± 4	39 ± 3	242 ± 16	238 ± 14	199 ± 23

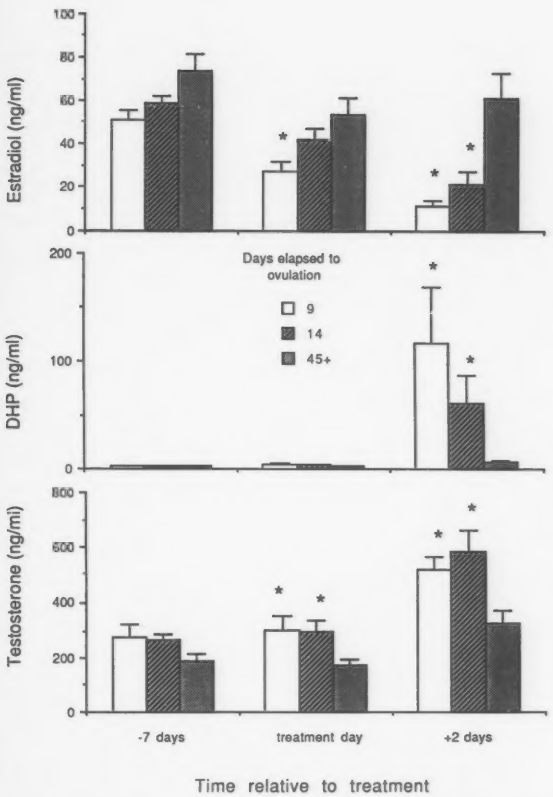


FIG. 3. Mean plasma concentrations of estradiol-17β, 17α,20β-dihydoxy-4-pregnen-3-one (DHP), and testosterone in female coho salmon before (−7 and 0 d) and after (+2 d) treatment with gonadotropin-releasing hormone analog (GnRHa). Each value represents the mean and standard error from females (n = 11 − 13) in groups differentiated by the number of days elapsed to ovulation from 22 October 1985 (0 d), i.e. 9, 14, or 45+ d after first treament. Means marked by an asterisk are significantly different from others on that particular day.

In 1985, no significant difference in the concentrations of DHP on T_{-7} was detected between groups injected with saline, but on T_0, DHP was higher in fish that ovulated after 14 d than in those that ovulated after 45+ d. On T_2, DHP tended ($p < 0.10$) to be higher in saline-injected fish that ovulated after 14 d than after 30 or 45+ d.

In GnRHa-treated females, plasma DHP was lower on T_{-7} than on T_0 or T_2 in all groups and lower on T_0 than on T_2 (Fig. 3). On both T_{-7} and T_0, there was no significant difference in plasma DHP between groups, but on T_2 DHP was higher in fish that ovulated after 9 or 14 d than after 45+ d (Fig. 3).

Estradiol-17β

In 1984, the levels of estradiol on T_0 (16 October) did not vary as a function of days elapsed to ovulation after treatment with either GnRHa or saline (Fig. 2). Plasma levels of estradiol in fish treated with GnRHa on T_0' (23 October) did not differ between days elapsed to ovulation; however, in saline-injected fish, plasma estradiol on T_0' was lower in fish that ovulated on day 10 than on day 34 or 41 and in fish that ovulated on day 14 than on day 21, 27, 34, or 41 (Fig. 2).

In 1985, there was no significant difference in plasma estradiol on T_{-7}, T_0, or T_2 between groups that ovulated 14, 30, or 45+ d after saline injection (Table 2). In females that ovulated by either 9 or 14 d after the first injection of GnRHa, estradiol levels were higher on T_{-7} than on T_0 or T_2 and higher on T_0 than on T_2 (Fig. 3). No significant temporal changes were observed in estradiol in fish that ovulated 45+ d after administration of GnRHa.

Plasma estradiol on T_{-7} was not significantly different between fish that ovulated after 9, 14, or 45+ d, but on T_0, it was lower in fish that ovulated after 9 d than in those that ovulated after 14 or 45+ d (Fig. 3). On T_2, estradiol was lower in fish ovulating after 9 or 14 d than after 45+ d.

Testosterone

On T_0 in 1984, testosterone was higher in saline-treated females that ovulated 17 d after injection than in fish that ovulated 24, 34, or 41 d after treatment and was higher in fish that ovulated on day 28 than in those that ovulated on day 34, 41, or 48 (Fig. 2). Testosterone was higher in GnRHa-treated females that ovulated on day 10 than on day 34, 41, or 48 and was higher in females that ovulated on day 17 than on day 41.

Testosterone on T_0' was higher in saline-injected fish that ovulated on day 10 than on day 34 or 41, higher in fish that ovulated on day 14 than on day 34, 41, or 49, and higher in fish that ovulated on day 21 than on day 34 or 41. Plasma testosterone was higher in GnRHa-treated females that ovulated on day 7 than on day 21 or 34 and was higher in fish that ovulated on day 10 than on day 34 or 49 (Fig. 2).

In 1985, testosterone was similar in all saline-injected groups on T_{-7}, but on T_0, it was higher in fish that ovulated after 14 d than after 45+ d. On T_2, testosterone levels tended ($p < 0.10$) to be higher in fish ovulating after 14 d than after 30 or 45+ d (Table 2).

In all groups treated with GnRHa, plasma testosterone was significantly higher on T_2 than on T_{-7} or T_0 (Fig. 3). Plasma testosterone was similar in all groups on T_{-7}, although it tended

to be higher ($p < 0.12$) in fish that ovulated after 9 or 14 d than after 45+ d. On T_0 and T_2, testosterone was higher in fish ovulating after 9 or 14 d than after 45+ d (Fig. 3).

Discussion

Elevated concentrations of testosterone in plasma were strongly correlated with early ovulation in both control and GnRHa-injected female coho salmon in both 1984 and 1985. Estradiol and DHP concentrations were not consistently related to ovulatory timing, although temporal changes in their concentrations during the sampling period in 1985 seemed to reflect ovulatory responsiveness.

Information about the hormonal status of female salmon could be useful in predicting ovulatory timing in hatchery populations. Coloration and historical dates of maturation are now the criteria typically used in hatcheries to predict when females will mature. Inasmuch as changes in the environment may lead to year-to-year variations in the timing of spawning runs and in coloration, these selection criteria may not be adequate. Final maturation and ovulation are under endocrine control. Oocyte maturation has been correlated with increased plasma DHP and testosterone and with decreased estradiol (Scott et al. 1983; Van Der Kraak et al. 1984; Fitzpatrick et al. 1986). Information on hormonal status may be especially important in situations where ovulation is to be induced by GnRH and its analogs, a technique that is often but not always successful (Fig. 1; Van Der Kraak et al. 1983, 1985; Crim and Glebe 1984; Fitzpatrick et al. 1984).

This variable success suggests that there is a limit to the effectiveness of GnRHa in accelerating ovulation which may be related to the length of time that treatment occurs before natural ovulation. Crim and Glebe (1984) demonstrated that in Atlantic salmon treated with a pelleted GnRH analog, only 30% of females ovulated when implanted 45 d before the spawning time of control fish, but 94% ovulated when the implantation was made 28 d before that spawning time. In the present study in 1984, 29% of the fish injected on 16 October and 52% of those injected on 23 October ovulated within 2 wk. Thus, delaying GnRHa treatment 1 wk increased the percentage of females that responded within 2 wk of treatment.

The value of plasma estradiol concentration as a predictive index was questionable: in 1984 the concentration varied as a function of ovulatory response in only one of four treatment regimes (saline injected, 23 October; Fig. 2), and in 1985, neither at 1 wk before nor on the day of treatment did estradiol differ significantly between fish that ovulated after 14 d and those that ovulated after 45+ d. Therefore, only in females ovulating 9 d after injection of GnRHa did information about estradiol levels on the day of treatment provide a clue as to how quickly the fish would ovulate after treatment. In this study, low estradiol suggested that the animals would ovulate soon after treatment, but higher estradiol levels meant only that ovulation would occur at some time between 14 and 45 d or more after treatment. Plasma estradiol declined between 15 and 22 October 1985 in saline- and GnRHa-injected fish, significantly in fish that ovulated within 14 d after treatment with GnRHa. Thus, although temporal changes in estradiol may have some predictive value, the predictive utility is lessened due to the requirement of multiple sampling.

DHP is of little use in predicting how the animals will respond to treatment. In salmonids, administration of gonadotropin in vivo raises plasma concentrations of DHP (Scott et al. 1982; Wright and Hunt 1982; Van Der Kraak et al. 1985), and in vitro incubation of ovaries with gonadotropin increases follicular production of DHP (Young et al. 1982, 1983; Jalabert and Fostier 1984). It has been demonstrated that various analogs of GnRH increase the plasma levels of gonadotropin in coho salmon (Van Der Kraak et al. 1983, 1985), Atlantic salmon (Crim et al. 1983a; Crim and Glebe 1984), and rainbow trout (Crim et al. 1983b). In the present study, plasma concentrations of DHP did not differ between early- and late-ovulating groups before treatment; however, by 2 d after the initial injection in 1985, levels of DHP were higher in fish that would ovulate by 9 or 14 d than in those that required 45 d or longer. This finding is consistent with those of Van Der Kraak et al. (1985) in coho salmon. Increases in DHP levels between 15 and 24 October 1985 in all groups prevented clear discrimination based on temporal changes.

In both 1984 and 1985, data on plasma testosterone concentration on the day of treatment with saline or GnRHa allowed clear discrimination between early- or late-ovulating fish. This relation tended ($p < 0.12$) to hold for samples taken 1 wk before treatment with GnRHa in 1985. Testosterone levels were also higher in early- than in late-ovulating fish 2 d after treatment. It is impossible to specify a particular plasma concentration of testosterone that will guarantee successful response to GnRHa. However, of the 49 GnRHa-treated females with plasma testosterone levels on the treatment date above 200 ng/mL (1984 and 1985 data combined), 81.6% ovulated within 2 wk of treatment. Conversely, of the 46 GnRHa-treated females with testosterone levels less than 150 ng/mL, only 19.6% ovulated within 2 wk. Thus, high testosterone levels indicate the likelihood of a successful response (i.e. ovulation within 2 wk) whereas lower levels indicate minimal response. Caution should be exercised when extending these results to other situations where genetic and environmental factors differ from those in the present study.

The function of testosterone in adult female salmon is unclear, although the predictive value of testosterone for responsivity to GnRHa suggests a functional relation. Androgens, including testosterone, enhance the synthesis and storage of gonadotropin in the pituitaries of juvenile salmonids (Crim and Evans 1979; Gielen et al. 1982; Van den Hurk et al. 1984). Crim and Evans (1980) demonstrated that in vivo pretreatment of immature rainbow trout with testosterone resulted in gonadotropin accumulation in the pituitary gland which was readily released in vitro upon stimulation with GnRH. Testosterone has also been shown to enhance the effect of gonadotropin on the in vitro maturation of oocytes in amago salmon, *O. rhodurus* (Nagahama et al. 1980; Young et al. 1982).

In view of the present results, it is plausible that the increased levels of testosterone in early-ovulating fish resulted in increased synthesis of gonadotropin by the pituitary. Upon stimulation by GnRHa, gonadotropin was probably released, causing increased production of DHP by the ovarian follicles. The decreasing output of estradiol may have permitted this action, as it has been shown that high levels of estradiol decrease the responsiveness of ovarian follicles of rainbow trout to gonadotropin (Jalabert and Fostier 1984). Testosterone is aromatized to estradiol-17β in the granulosa layer of the ovarian follicles of amogo salmon (Kagawa et al. 1982). It is not known what effect testosterone has on aromatization activity during this stage of maturation. Further investigation is required to determine if the increase in plasma testosterone levels is a cause or an effect of the decrease in circulating estradiol.

The results of our study demonstrate the effectiveness of GnRHa in accelerating maturation in coho salmon. These findings are consistent with those in previous studies (Van Der Kraak et al. 1983, 1985; Fitzpatrick et al. 1984). Although GnRHa represents an effective tool in the management of hatchery brood stock populations, it does not accelerate maturation in all treated females in a given hatchery population. Therefore, to improve the efficiency of GnRHa treatment, the fish should be screened before treatment to select the animals most likely to respond, or alternatively, treatment should be postponed until most of the animals can be expected to respond favorably. Measurement of the circulating levels of testosterone appears to be a promising method for such screening of female coho salmon.

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References

CRIM, L. W., AND D. M. EVANS. 1979. Stimulation of pituitary gonadotropin by testosterone in juvenille rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 37: 192–196.

1980. LH-RH-stimulated gonadotropin release from the rainbow trout pituitary gland: an in vitro assay for detection of teleost gonadotropin releasing factor(s). Gen. Comp. Endocrinol. 40: 283–290.

CRIM, L. W., D. M. EVANS, AND B. H. VICKERY. 1983a. Manipulation of the seasonal reproductive cycle of landlocked Atlantic salmon (*Salmo salar*) by LHRH analogues administered at various stages of gonadal development. Can. J. Fish. Aquat. Sci. 40: 61–67.

CRIM, L. W., AND B. D. GLEBE. 1984. Advancement and synchrony of ovulation in Atlantic salmon with pelleted LHRH analog. Aquaculture 43: 47–56.

CRIM, L. W., A. M. SUTTERLIN, D. M. EVANS, AND C. WEIL. 1983b. Accelerated ovulation by pelleted LHRH analogue treatment of spring-spawning rainbow trout (*Salmo gairdneri*) held at low temperature. Aquaculture 35: 299–307.

FITZPATRICK, M. S., B. K. SUZUMOTO, C. B. SCHRECK, AND D. OBERBILLIG. 1984. Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). Aquaculture 43: 67–73.

FITZPATRICK, M. S., G. VAN DER KRAAK, AND C. B. SCHRECK. 1986. Profiles of plasma sex steroids and gonadotropin in coho salmon, *Oncorhynchus kisutch*, during final maturation. Gen. Comp. Endocrinol. 62: 437–451.

GIELEN, J. T., H. J. T. GOOS, J. PEUTE, R. A. VAN DEN BOSCH, AND P. G. W. J. VAN OORDT. 1982. The brain–pituitary–gonadal axis in the rainbow trout, *Salmo gairdneri*: gonadal hormones and the maturation of gonadotropic cells. Cell Tissue Res. 225: 45–56.

GOETZ, F. W. 1983. Hormonal control of oocyte final maturation and ovulation in fishes, p. 117–170. *In* W. S. Hoar, D. J. Randall, and E. M. Donaldson [ed.] Fish physiology. Vol. 9B. Academic Press, New York, NY.

JALABERT, B. 1976. In vitro oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). J. Fish. Res. Board Can. 33: 974–988.

JALABERT, B., AND A. FOSTIER. 1984. The follicular sensitivity in vitro to maturation-inducing hormones in rainbow trout, *Salmo gairdneri*: role of oestradiol-17β. Aquaculture 43: 1–11.

KAGAWA, H., G. YOUNG, S. ADACHI, AND Y. NAGAHAMA. 1982. Estradiol-17β production in amago salmon (*Oncorhynchus rhodurus*) ovarian follicles: role of thecal and granulosa cells. Gen. Comp. Endocrinol. 47: 440–448.

NAGAHAMA, Y., H. KAGAWA, AND F. TASHIRO. 1980. The in vitro effects of various gonadotropins and steroid hormones on oocyte maturation in amago salmon *Oncorhynchus rhodurus* and rainbow trout *Salmo gairdneri*. Bull. Jpn. Soc. Sci. Fish. 46: 1097–1102.

SCOTT, A. P., E. L. SHELDRICK, AND A. P. FLINT. 1982. Measurement of 17α,20β-dihydroxy-4-pregnen-3-one in plasma of trout (*Salmo gairdneri* Richardson): seasonal changes and response to salmon pituitary extract. Gen. Comp. Endocrinol. 46: 444–451.

SCOTT, A. P., J. P. SUMPTER, AND J. P. HARDIMAN. 1983. Hormone changes during ovulation in rainbow trout (*Salmo gairdneri* Richardson). Gen. Comp. Endocrinol. 49: 128–134.

SHERWOOD, N., L. EIDEN, M. BROWNSTEIN, J. SPIESS, J. RIVIER, AND W. VALE. 1983. Characterization of a teleost gonadotropin-releasing hormone. Proc. Natl. Acad. Sci. USA 80: 2794–2798.

SOWER, S. A., R. N. IWAMOTO, W. W. DICKHOFF, AND A. GORBMAN. 1984. Ovulatory and steroidal responses in coho salmon and steelhead trout following administration of salmon gonadotropin and D-Ala^6,des-Gly^{10} gonadotropin-releasing hormone ethylamide (GnRHa). Aquaculture 43: 35–46.

VAN DEN HURK, R., J. TH. GIELEN, AND M. TERLOU. 1984. Accumulation of glycoprotein gonadotropin in the pituitary of juvenile rainbow trout in response to androgens and C21-steroids, including 11-steroids. Cell Tissue Res. 235: 635–642.

VAN DER KRAAK, G., H. M. DYE, AND E. M. DONALDSON. 1984. Effects of LH-RH and des-Gly^{10}[D-Ala^6]LH-RH-ethylamide on plasma sex steroid profiles in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 55: 36–45.

VAN DER KRAAK, G., H. M. DYE, AND E. M. DONALDSON, AND G. A. HUNTER. 1985. Plasma gonadotropin, 17β-estradiol, and 17α,20β-dihydroxy-4-pregnen-3-one levels during luteinizing hormone-releasing hormone analogue and gonadotropin induced ovulation in coho salmon (*Oncorhynchus kisutch*). Can. J. Zool. 63: 824–833.

VAN DER KRAAK, G., H.-R. LIN, E. M. DONALDSON., H. M. DYE, AND G. A. HUNTER. 1983. Effects of LH-RH and des-Gly^{10}[D-Ala^6]LH-RH-ethylamide on plasma gonadotropin levels and oocyte maturation in adult female coho salmon (*Oncorhynchus kisutch*). Gen. Comp. Endocrinol. 49: 470–476.

WRIGHT, R. S., AND S. M. V. HUNT. 1982. A radioimmunoassay for 17α,20β-dihydroxy-4-pregnen-3-one: its use in measuring changes in serum levels at ovulation in Atlantic salmon (*Salmo salar*), coho salmon (*Oncorhynchus kisutch*), and rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 47: 475–482.

YOUNG, G., L. W. CRIM, H. KAGAWA, A. KAMBEGAWA, AND Y. NAGAHAMA. 1983. Plasma 17α,20β-dihydroxy-4-pregnen-3-one levels during sexual maturation of amago salmon (*Oncorhynchus rhodurus*): correlation with plasma gonadotropin and in vitro production by ovarian follicles. Gen. Comp. Endocrinol. 51: 96–105.

YOUNG, G., H. KAGAWA, AND Y. NAGAHAMA. 1982. Oocyte maturation in the amago salmon (*Oncorhynchus rhodurus*): in vitro effects of salmon gonadotropin, steriods, and cyanoketone (an inhibitor of 3β-hydoxy-Δ^5-steriod dehydrogenase). J. Exp. Zool. 224: 265–275.

NOTES

Effect of Surface Access and Oxygen Concentration on the Toxicity of *Croton tiglium* (Euphorbiaceae) Seed Extract to *Brachydanio rerio* (Cyprinidae)

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Kulakkattolickal, A. T., and D. L. Kramer. 1987. Effect of surface access and oxygen concentration on the toxicity of *Croton tiglium* (Euphorbiaceae) seed extract to *Brachydanio rerio* (Cyprinidae). Can. J. Fish. Aquat. Sci. 44: 1358–1361.

Zebrafish (*Brachydanio rerio*) (Cyprinidae) were exposed to a toxic extract of *Croton tiglium* (Euphorbiaceae) seeds (4 $mg \cdot L^{-1}$) at 0.8, 1.1 and 8.1 mg $O_2 \cdot L^{-1}$, with and without surface access. Equilibrium loss occurred more quickly at lower dissolved oxygen concentrations. At 0.8 and 1.1 mg $O_2 \cdot L^{-1}$, equilibrium loss also occurred more quickly when access to the water surface was denied. Surface access is therefore an important factor in studies of the interaction between toxicity and dissolved oxygen concentration. We suggest that, by performing aquatic surface respiration, zebrafish with surface access in hypoxic water reduce their ventilation rate and hence their uptake rate of toxins.

On a exposé des zèbres (*Brachydanio rerio*) (Cyprinidae) à des extraits toxiques (0,8, 1,1 et 8,1 $mg \cdot L^{-1}$ de O_2) de graines (4 $mg \cdot L^{-1}$) de *Croton tiglium* (Euphorbiaceae) dans un milieu où les poissons avaient accès ou non à la surface. La perte d'équilibre a eu lieu plus rapidement aux faibles concentrations d'oxygène dissous. À 0,8 et 1,1 $mg \cdot L^{-1}$ de O_2, la perte d'équilibre s'est aussi produite plus rapidement quand le poisson n'avait pas accès à la surface. L'accès à la surface représente donc un important facteur dans les études sur l'interaction entre la toxicité et la concentration d'oxygène dissous. Les auteurs avancent la conclusion que les zèbres qui ont accès à la surface en milieu hypoxique réduisent leur taux de ventilation et donc leur taux d'absorption de toxines quand ils respirent à la surface.

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A wide range of chemical pollutants are more toxic to fishes at low levels of dissolved oxygen than at higher levels. Cyanide (Southgate et al. 1933; Downing 1954), ammonia (Lloyd 1961; Thurston et al. 1981), hydrogen sulfide (Smith and Oseid 1972), phenols (Lloyd 1961; Gupta et al. 1983), heavy metals (Pickering 1968; Voyer et al. 1975; Verma et al. 1985), and kraft mill effluent (Hicks and DeWitt 1971) all cause mortality at lower concentrations or in less time under hypoxic conditions than under well-oxygenated ones. Low levels of dissolved oxygen may increase toxicity by increasing ventilation rate which increases the flux of toxicant over the gill epithelium, a major site of absorption (Lloyd 1961). It is therefore surprising that little attention has been given to the interaction between surface access and hypoxia in toxicological studies.

Under hypoxic conditions, most non-air-breathing fish species rise to the air–water interface where they perform aquatic surface respiration, inspiring the oxygen-rich surface layer maintained by diffusion (Lewis 1970; Gee et al. 1978; Kramer and McClure 1982). Aquatic surface respiration increases survival, growth, activity, and blood oxygen levels under hypoxic conditions (Lewis 1970; Kramer and Mehegan 1981; Burggren 1982; Weber and Kramer 1983) and probably permits the maintenance of oxygen uptake at lower ventilation rates (Gee et al. 1978). Thus, surface access may improve survival in the presence of toxicants, but no study has experimentally examined the effect of surface access on toxicity, and most investigations of the interaction between oxygen concentration and toxicity have even failed to record whether or not there was access to the surface. The present study was designed to examine the interaction between surface access and oxygen concentration on the toxicity of a waterborne substance to a fish with exclusively aquatic respiration. As a toxin we used an extract of the seeds of *Croton tiglium* (Euphorbiaceae) which is used in India to harvest wild fish for human consumption (A. T. Kulakkattolickal, unpubl. obs.) and has been proposed as a substitute for rotenone to remove predators and competitors before stocking aquaculture ponds (Bhuyan 1968; Jhingran 1975, p. 550–554).

Materials and Methods

Croton tiglium seeds were imported from Kerala, India. The seed coats were removed, and 90 g of the remaining portion was ground into a fine powder in liquid nitrogen using a mortar

TABLE 1. Analysis of variance for equilibrium loss time of *Brachydanio* in relation to oxygen, toxin and surface access.

Source	Mean square	*F* ratio	df	Significance
Oxygen	41976.867	26.944	2	$p < 0.001$
Toxin	300900.017	193.142	1	$p < 0.001$
Access	32994.150	21.178	1	$p < 0.001$
Oxygen × toxin	19060.467	12.235	2	$p < 0.001$
Oxygen × access	7284.200	4.676	2	$p = 0.014$
Toxin × access	8906.017	5.717	1	$p = 0.021$
Oxygen × toxin × access	3746.467	2.405	2	ns
Residual	1557.925			

and pestle and then homogenized in a small amount of water, suspended over four layers of bleached cheese cloth (Fisher grade No. 50), and extracted with 9 L of water to give a 1% (weight/volume) stock solution. Aliquots of 30 mL were frozen and then thawed at room temperature as needed.

As a test species, we used 300 zebrafish (*Brachydanio rerio*) (Cyprinidae) purchased from a local aquarium wholesaler (standard length 29.42 ± 2.43 mm, weight 390.3 ± 105.3 mg ($\bar{x}$ ± SD)). This species is widely used in toxicological testing (Laale 1977) and exhibits aquatic surface respiration (Lewis 1970).

Tests were conducted in aquaria measuring 76 × 30 × 30 cm deep divided into two equal sections by a plastic screen perforated by 1-mm-diameter holes at 5-mm intervals. On one side, Plexiglas supports were provided for a glass cover that could be positioned to prevent surface access. Adjacent to each aquarium was a 3.5-L jar in which oxygen levels (0.8, 1.1, and 8.1 mg $O_2 \cdot L^{-1}$) were controlled by bubbling in nitrogen or air. Water was pumped from the jar into both sides of the aquarium at a rate of 4 $L \cdot min^{-1}$ and siphoned back into the jar. The entire apparatus contained a volume of 64 L. Oxygen levels on the two sides of the aquarium were equalized by small adjustments in the flow rates to each side. Tests using dyes verified that the water was well mixed. Water temperature was maintained at 25 ± 0.6°C. Siphons in each half of the aquarium were used to collect water samples into a 250-mL graduated cylinder for oxygen measurement using a Yellow Springs Instruments model 57 oxygen meter. For hypoxic trials the cylinder was filled with nitrogen gas before use to reduce oxygenation of the sample. All readings were corrected for changes during collection, using a previously determined calibration curve relating aquarium dissolved oxygen to cylinder reading.

Five trials were conducted at each nominal oxygen concentration. Each trial involved one tank with and one tank without toxin, each tank having one section with and one without surface access. Two days before each trial, fish were fin-clipped to permit individual identification. The experimental tanks were set up on the evening before the trial and filled with aged tap water. On the morning of the trial, five marked fish were randomly allocated to each half of both tanks and left (with surface access) for 4–5 h until the desired oxygen level was achieved. Then, 25.6 mL of the stock solution was added to the experimental aquarium equally divided between both sides, to provide a 4 mg $\cdot L^{-1}$ concentration of toxin, and the glass covers were put in place. The fish were observed continuously for the next 5 h and the equilibrium loss time (ELT) of each individual was noted. Equilibrium loss was defined by the failure of fish to maintain their dorso-ventral body axis in the vertical plane. It was used as an indicator of mortality because of the difficulty of making more detailed observations of opercular or other movements on so many individuals. In preliminary experiments, fish that reached this stage never recovered upon transfer to freshwater.

Oxygen was measured at intervals of 1 h. A two-way analysis of variance (Sokal and Rohlf 1981) revealed no significant effect of surface access or toxin treatments or their interaction on oxygen level. Variation among trials in mean oxygen level was relatively small. Means ± SD and ranges for very hypoxic trials were 0.8 ± 0.1 mg $O_2 \cdot L^{-1}$ (0.6–1.0 mg $O_2 \cdot L^{-1}$), for moderately hypoxic trials 1.1 ± 0.1 mg $O_2 \cdot L^{-1}$ (1.0–1.3 mg $O_2 \cdot L^{-1}$), and for normoxic trials 8.1 ± 0.2 mg $O_2 \cdot L^{-1}$ (7.7–8.3 mg $O_2 \cdot L^{-1}$). A detailed summary of oxygen variations is given in Kulakkattolickal (1986). Tanks were detoxified after each trial by circulating in them overnight an 8 mg $\cdot L^{-1}$ solution of potassium permanganate.

For each treatment in each trial the median ELT in minutes was determined. Then a three-way analysis of variance (Sokal and Rohlf 1981) was performed on the median values of ELT to examine the effects of the presence or absence of toxin, presence or absence of surface access, level of dissolved oxygen, and their interactions.

Results and Discussion

Toxin, surface access, oxygen concentration, and their two-way interactions all had significant effects on ELT (Table 1). With surface access and no toxin the median fish survived the test period at all oxygen levels. With surface access and toxin the median fish lost equilibrium before the test finished in all but one trial, and ELT decreased as oxygen concentration decreased (Fig. 1). Without surface access or toxin the median fish survived all trials at 8.0 and 1.1 mg $O_2 \cdot L^{-1}$ but died before the end of three of five trials at 0.8 mg $O_2 \cdot L^{-1}$. Without surface access but with toxin the equilibrium loss of the median fish always occurred before the end of the trial, and again ELT decreased as oxygen concentration decreased (Fig. 1). In addition, the ELTs of fish in toxin were consistently shorter without surface access than with surface access at both 0.8 and 1.1 mg $O_2 \cdot L^{-1}$, but not at 8.1 mg $O_2 \cdot L^{-1}$ (Fig. 1). Observations during the first 30 min of treatment revealed that the fish spent over 60% of their time in contact with the surface in both toxin and control tests at 0.8 and 1.1 mg $O_2 \cdot L^{-1}$.

The present experiment confirms earlier findings that toxicity of waterborne substances to fishes increases as oxygen decreases (see the introduction for references). Previous studies have primarily involved heavy metals and small molecules or ions such as ammonia and cyanide. Ours is the first record for a plant toxin. These toxins apparently include proteins but have not been completely characterized (Kraemer 1915, p. 475–476; Stirpe et al. 1976; Banerjee and Sen 1981).

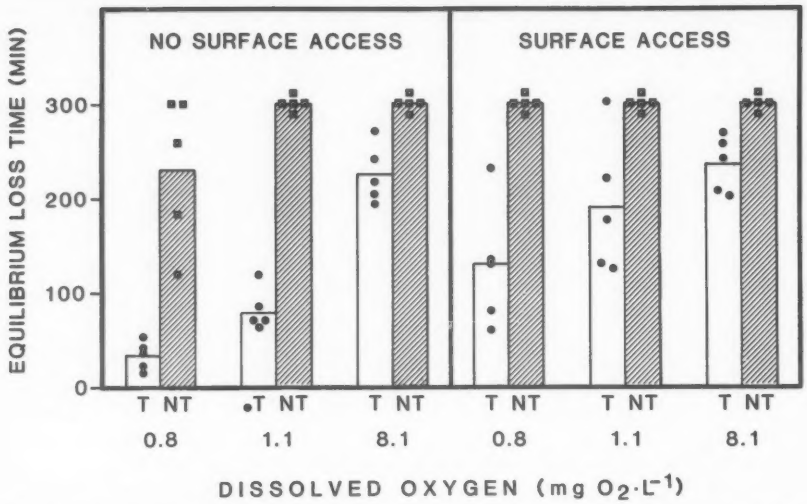

FIG. 1. Comparison of equilibrium loss time of *Brachydanio* in toxin (T, open bars) and no toxin (NT, hatched bars) with and without surface access at three dissolved oxygen levels. Circles and squares represent the median values of survival time of five groups of five fish in toxin and no toxin, respectively. Each bar represents the mean of the medians; points clustered around the top of the bars indicate that the fish survived for the entire experimental period of 300 min.

Our study also provides the first direct evidence that surface access affects toxicity under hypoxic conditions. Not only did the fish in toxin live significantly longer when they had surface access than when they did not, but the lack of access accentuated the effect of oxygen levels on toxicity. This magnification of the effect of hypoxia by the lack of surface access parallels previous studies of the effect of surface access on growth and activity (Weber and Kramer 1983). In a companion study (Kulakkattolickal and Kramer 1987), we found that lack of access also increased the toxicity of *Croton* extract to the facultative air-breathing fish *Clarias macrocephalus*.

Our results help to interpret a previous study of an apparent age-related effect of oxygen on toxicity. Graves et al. (1981) reported an effect of oxygen on the toxicity of kraft mill effluent to juvenile sheepshead minnows (*Cyprinodon variegatus*), although no effect on fry or adults was observed. Interestingly, only the test containers used for juveniles apparently lacked surface access.

The evidence from our study supports the conclusion that, in addition to reducing the cost of ventilation, a reduction in toxin uptake may be a benefit of performing aquatic surface respiration. It is very important that future investigations of the effect of hypoxia on fishes control and report surface access. In general, laboratory studies preventing surface access are likely to find more severe effects of hypoxia than may occur under natural conditions where fish can perform aquatic surface respiration.

The generality of the effect of oxygen on toxicity and the evidence that this effect is compounded by lack of surface access may have application to aquaculture because many fish-rearing ponds show strong diurnal oxygen fluctuations (Hickling 1971, p. 48; Jhingran 1975, p. 356–357; Woynarovich 1975, p. 19). On the one hand, low oxygen levels can exacerbate the effects of contaminants in the water supply, but on the other hand, they could reduce the dose of toxicant used to remove unwanted fish from aquaculture ponds before stocking if toxicants were applied when oxygen levels are minimal. In addition or alternatively, access to the surface could be reduced by using a barrier such as plastic sheeting, or even by frightening the fish away from the surface. The interaction of oxygen and surface access is an important process which should be considered in both laboratory and field studies involving toxicants.

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References

BANERJEE, K. K., AND A. SEN. 1981. Purification and properties of a lectin from the seeds of *Croton tiglium* with hemolytic activity toward rabbit red cells. Arch. Biochem. Biophys. 212: 740–753.

BHUYAN, B. R. 1968. A note on the use of *Croton tiglium* Linn. seed as a fish poison in ponds. J. Bombay Nat. Hist. Soc. 65: 236–240.

BURGGREN, W. W. 1982. Air gulping improves blood oxygen transport during aquatic hypoxia in goldfish, *Carassius auratus*. Physiol. Zool. 55: 327–334.

DOWNING, K. M. 1954. The influence of dissolved oxygen concentration on the toxicity of potassium cyanide to rainbow trout. J. Exp. Biol. 31: 161–164.

GEE, J. H., R. F. TALLMAN, AND H. J. SMART. 1978. Reactions of some great plains fishes to progressive hypoxia. Can. J. Zool. 56: 1962–1966.

GRAVES, W. C., D. T. BURTON, L. B. RICHARDSON, AND S. L. MARGREY. 1981. The interaction of treated bleached kraft mill effluent and dissolved oxygen concentration on the survival of the developmental stages of the sheepshead minnow (*Cyprinodon variegatus*). Water Res. 15: 1005–1011.

GUPTA, S., R. C. DALELA, AND P. K. SAXENA. 1983. Influence of dissolved oxygen levels on acute toxicity of phenolic compounds to fresh water teleost, *Notopterus notopterus* (Pallas). Water Air Soil Pollut. 19: 223–228.

HICKLING, C. F. 1971. Fish culture. 2nd ed. Faber and Faber, London. 317 p.

HICKS, D. B., AND J. W. DEWITT. 1971. Effects of dissolved oxygen on kraft pulp mill effluent toxicity. Water Res. 5: 693–701.

JHINGRAN, V. G. 1975. Fish and fisheries of India. Hindustan Publishing Corporation (India), Delhi. 954 p.

KRAEMER, H. 1915. Scientific and applied pharmacognosy for students of pharmacy and practicing pharmacists, food and drug analysts and pharmacologists. John Wiley & Sons, New York, NY. 857 p.

KRAMER, D. L., AND M. MCCLURE. 1982. Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fishes. Environ. Biol. Fishes 7: 47–55.

KRAMER, D. L., AND J. P. MEHEGAN. 1981. Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (Pisces, Poeciliidae). Environ. Biol. Fishes 6: 229–313.

KULAKKATTOLICKAL, A. T. 1986. The effects of surface access and dissolved oxygen levels on survival time of a water-breathing and an air-breathing fish species exposed to a plant toxin (*Croton tiglium*, Euphorbiaceae, seed extract). M.Sc. thesis, McGill University, Montreal, Que.

KULAKKATTOLICKAL, A. T., AND D. L. KRAMER. 1987. The role of air breathing in the resistance of bimodally respiring fish to waterborne toxins. J. Fish Biol. (In press).

LAALE, H. W. 1977. The biology and use of zebrafish, *Brachydanio rerio* in fisheries research. A literature review. J. Fish Biol. 10: 121–173.

LEWIS, W. M. JR. 1970. Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. Copeia 1970: 319–326.

LLOYD, R. 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). J. Exp. Biol. 38: 447–455.

PICKERING, Q. H. 1968. Some effects of dissolved oxygen concentrations upon the toxicity of zinc to the bluegill, *Lepomis macrochirus*, Raf. Water Res. 2: 187–194.

SMITH, L. L., AND D. M. OSEID. 1972. Effects of hydrogen sulfide on fish eggs and fry. Water Res. 6: 711–720.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Co., New York, NY. 859 p.

SOUTHGATE, B. A., F. T. K. PENTELOW, AND R. BASSINDALE. 1933. The toxicity to trout of potassium cyanide and *p*-cresol in water containing different concentrations of dissolved oxygen. Biochem. J. 27: 983–985.

STIRPE, F. A., A. PESSION-BRIZZI, E. LORENZONI, P. STROCCHI, L. MONTANARO, AND S. SPERTI. 1976. Studies on the proteins from the seeds of *Croton tiglium* and of *Jatropha curcas*. Toxic properties and inhibition of protein synthesis *in vitro*. Biochem. J. 1556: 1–6.

THURSTON, R. V., G. R. PHILLIPS, R. C. RUSSO, AND S. M. HINKINS. 1981. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Can. J. Fish. Aquat. Sci. 38: 983–988.

VERMA, S. R., R. CHAND, AND I. P. TONK. 1985. Effects of environmental and biological variables on the toxicity of mercuric chloride. Water Air Soil Pollut. 25: 243–248.

VOYER, R. A., P. O. YEVICH, AND C. A. BARSZCZ. 1975. Histological and toxicological responses of the mummichog, *Fundulus heteroclitus* (L.) to combinations of levels of cadmium and dissolved oxygen in a freshwater. Water Res. 1069–1074.

WEBER, J.-M., AND D. L. KRAMER. 1983. Effects of hypoxia and surface access on growth, mortality, and behavior of juvenile guppies, *Poecilia reticulata*. Can. J. Fish. Aquat. Sci. 40: 1583–1588.

WOYNAROVICH, E. 1975. Elementary guide to fish culture in Nepal. Food and Agriculture Organization of the United Nations, Rome. 131 p.

Identification of Planktonic Sea Scallop Larvae (*Placopecten magellanicus*) (Gmelin)

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Methods for the collection, processing, and identification of planktonic sea scallop larvae (*Placopecten magellanicus*) (Gmelin) are described. Bivalve larvae collected from the Bay of Fundy were compared with cultured *P. magellanicus* larvae. Sea scallop larvae collected from the plankton can be tentatively identified based on shape and size; examination of the larval hinge structure allows confirmation.

On présente des méthodes pour la cueillette, la préparation et l'identification des larves planctoniques du pétoncle géant (*Placopecten magellanicus*) (Gmelin). Les larves recueillies dans la baie de Fundy sont comparées aux larves d'élevage de *P. magellanicus*. Les premières peuvent être identifiées expérimentalement d'après la forme et la taille; l'examen de la structure de la charnière permet de confirmer l'identification.

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The sea scallop (*Placopecten magellanicus*) (Gmelin) is of considerable commercial importance to both Canada and the United States. For fishery management, the distribution and biology of the larval stage are important for two reasons: (1) if genetic exchange between commercial concentrations of adults occurs, it is likely to occur during the pelagic larval stage; and (2) mortality is probably greatest during the larval period. Both Dickie (1955) and Caddy (1979)

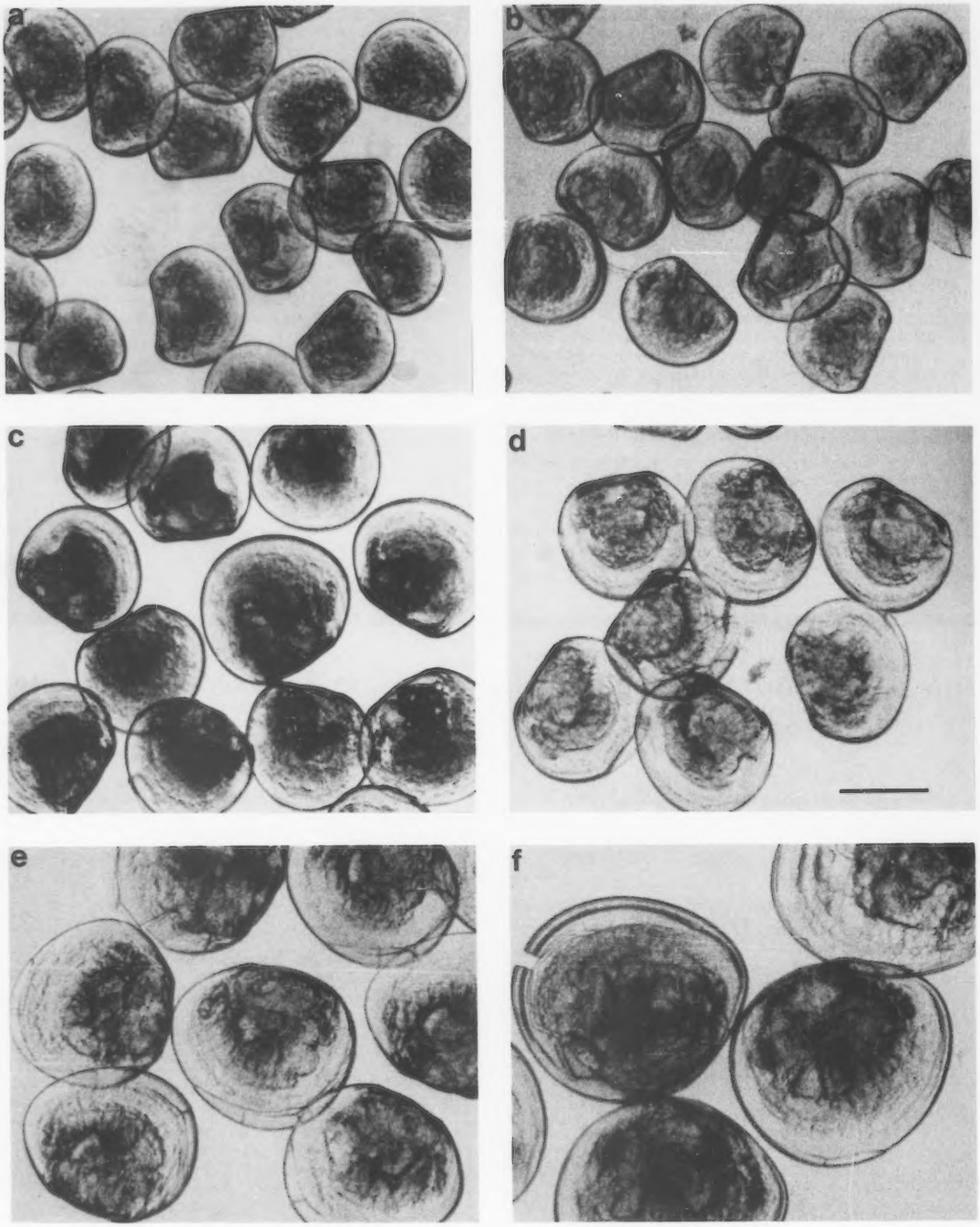

FIG. 1. (a and c) Cultured and (b, d, e, and f) wild sea scallop larvae photographed through a light microscope. Cultured larvae are 10 and 28 d old. Wild larvae were collected in the Bay of Fundy in October 1985. Scale bar = 100 μm.

have suggested that environmental factors acting during the larval period affect the size of the year class which is recruited to the fishery.

In spite of the importance of the larval stage for understanding the population dynamics of the sea scallop, studies of its distribution and biology are practically nonexistent (MacKenzie 1979). The only published account with micrographs of all larval stages is from the laboratory experiments of Culliney

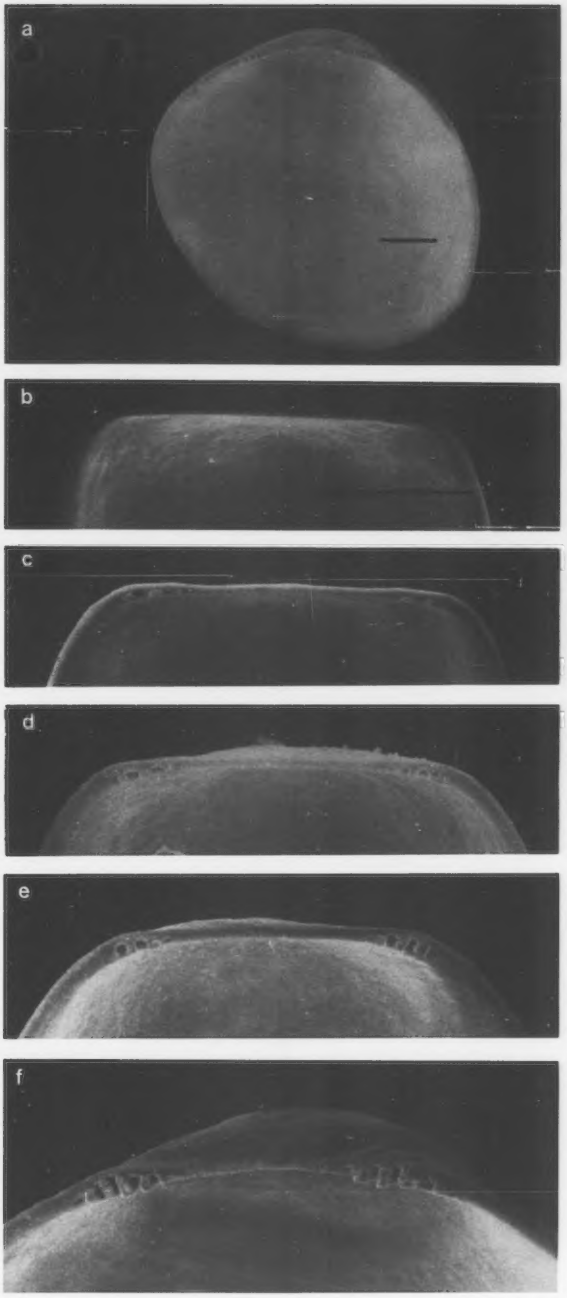

FIG. 2. SEM micrographs of hinge structure of cultured sea scallop larvae. (a) 35 d, right valve (r.v.); (b) 4 d, length (lng) = 112 μm, left valve (l.v.); (c) 7 d, lng = 119 μm, r.v.; (d) 18 d, lng = 139 μm, r.v.; (e) 22 d, lng = 150 μm, l.v.; (f) close-up of hinge of larva in Fig. 2a. Scale bar = 40 μm (Fig. 2b–2f at same scale).

(1974). There are also micrographs of larvae in Merrill (1961) and Bourne (1964). Larval sea scallop distribution has been documented only in a short communication based on samples

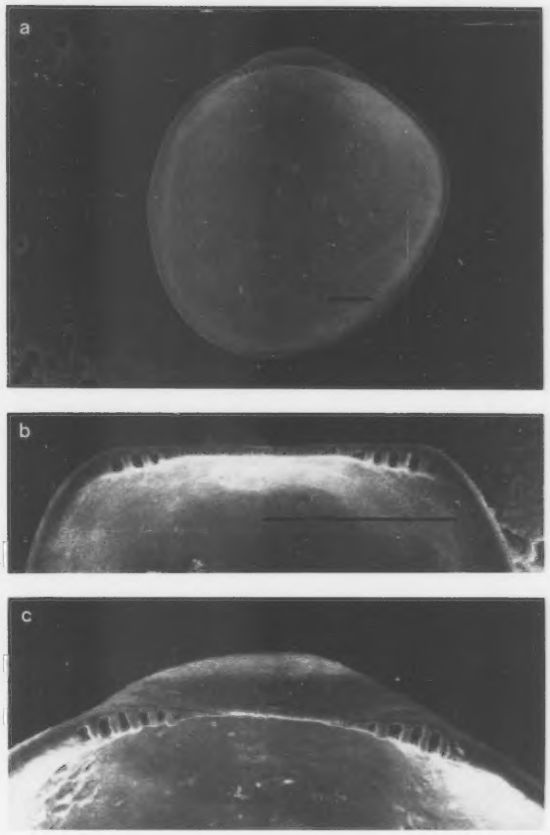

FIG. 3. SEM micrographs of hinge structure of wild sea scallop larvae collected in the Bay of Fundy in October 1985. (a) Left valve (l.v.); (b) length = 138 μm, l.v.; (c) close-up of hinge of larva in Fig. 3a. Scale bar = 40 μm (Fig. 3b and 3c at same scale).

from coastal New Hampshire (Savage 1980). As recently as 1979, Serchuk et al. (1979) (citing Bourne 1964) stated that *P. magellanicus* larvae had never been positively identified in plankton collections. Stafford (1912) reported that he had identified the larvae of sea scallops in "plankton collections from St. Andrews to Seven Islands," but Merrill (1961) showed that this identification was incorrect.

One reason for the lack of field studies may be the difficulty in identifying bivalve larvae to species. Larval sea scallops (and larval bivalves in general) are small (less than 300 μm) with few external distinguishing features. Nevertheless, the shape and size of *P. magellanicus* larvae are sufficiently characteristic to separate them from other bivalve larvae with which they co-occur in the plankton. Evidently, Savage (1980) also found this to be the case, but his purpose was to report on the total larval bivalve community, and his methods for distinguishing sea scallop larvae were not included.

A key distinguishing feature for the identification of bivalve larvae is larval hinge structure. This allows positive identification to the level of family at least, and in some cases to species (Le Pennec 1980; Lutz et al. 1982; Dominguez and Alcaraz 1983). The larval hinge structure of sea scallops has not been well described. Culliney (1974) provided an excellent descrip-

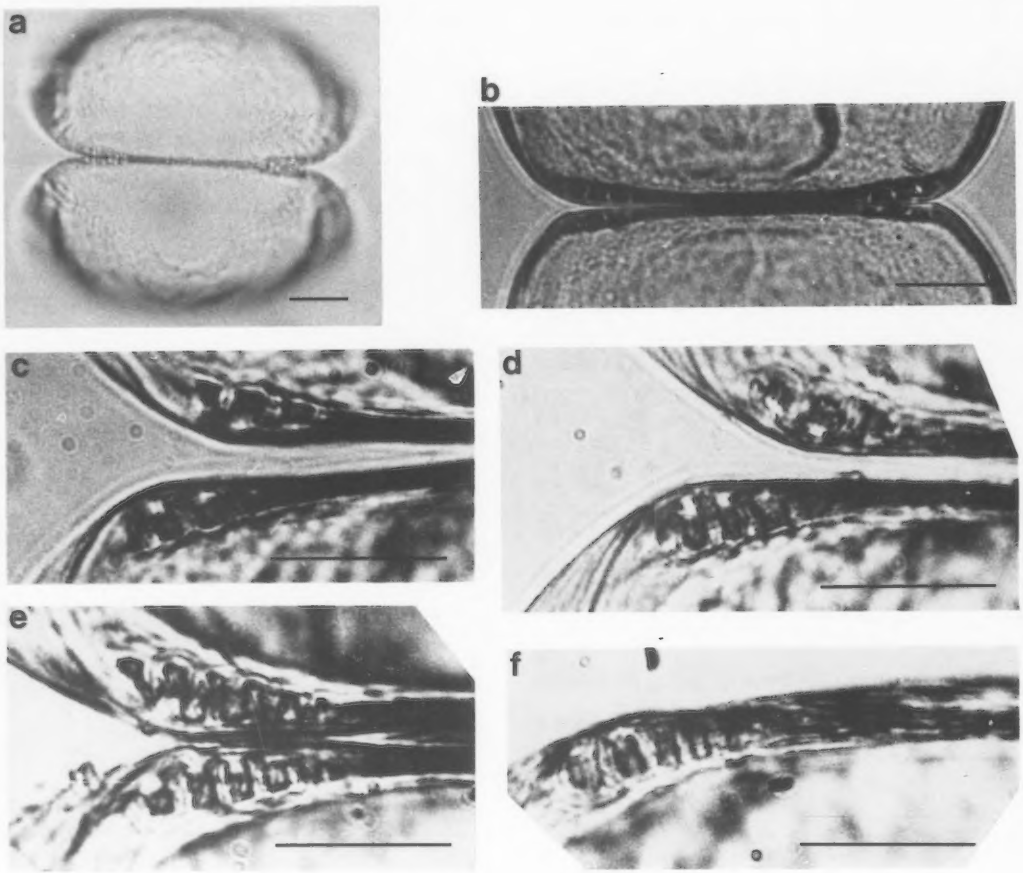

FIG. 4. Light micrographs of hinge structure of sea scallop larvae. All are wild larvae except for Fig. 4a. (a) Interior view of 7-d-old cultured larva (cf. Fig. 2c); (b) length (lng) = 118 μm; (c) lng = 130 μm; (d) lng = 160 μm; (e) lng = 190 μm; (f) lng = 250 μm. Scale bar = 20 μm.

tion of *P. magellanicus* larval development but the hinge structure was given little attention. There are two micrographs of a single *P. magellanicus* larval hinge available in the literature (Lutz et al. 1982), but these are insufficient for taxonomic purposes.

Herein, new methods for the physical and taxonomic separation of scallop larvae collected from the plankton are described. We also include micrographs of the hinge structure of sea scallop larvae of a broad size range. These will be useful for future investigators wishing to distinguish this species from other planktonic bivalve larvae.

Methods

Plankton samples were obtained from the Bay of Fundy in September, October, and November in 1984 and during October in 1985. Examination of adult gonads obtained from the commercial fishery in those years indicated that peak spawning occurred in late August and early September (G. Robert, Department of Fisheries and Oceans, Biological Sciences Branch, P.O. Box 550, Halifax, N.S., pers. comm.). Samples were collected with both a high-volume pump and plankton nets mounted on a bongo frame (net diameter either 40 or 50 cm). Mesh sizes were 64, 85, or 120 μm. Volumes sampled by the pump were generally 2–4 m^3; net volumes were anywhere from 5 to 45 m^3 depending on the depth of the water column. Samples were preserved in 4% buffered formalin.

The high specific gravity of bivalve larvae was used to separate them from the phytoplankton and crustacean components of the plankton samples. Whole samples were concentrated on a 64-μm screen and then backwashed with approximately 20 mL of water into a 125-mL separatory funnel containing Ludox® AM, a colloidal silica with a specific gravity of 1.2. Bivalve larvae (and other heavy components) then sank to the bottom of the funnel where they could be drained off.

Cultured scallop larvae were obtained from different batches reared in the Department of Biology and the Aquatron Laboratory of Dalhousie University, Halifax, N.S. The culture conditions are described in Couturier (1986) and Hurley et al. (1987). Generally, larvae were reared in 20-L buckets at a

temperature of 14°C and were fed a mixed algal diet. The cultured larvae were preserved in 95% ethanol.

To examine larval hinge structure, the valves were disarticulated by immersion in a 5% solution of commercial bleach (6% sodium hypochlorite) for 5–15 min. Hinges were photographed under both a scanning electron microscope (SEM) and an inverted light microscope. Procedures for SEM preparation were those of Lutz et al. (1982). Larval lengths were measured as the greatest distance parallel to the hinge.

Results and Discussion

The use of LUDOX® to separate the bivalve component from the remainder of the plankton sample made sample processing more efficient and less arduous. There was little difficulty in separating sea scallop larvae from other bivalve larvae once the phytoplankton and crustacean components were removed. Sea scallop larvae have a more pointed anterior end, a less robust shell, and a lighter colour than the mussel larvae (both *Mytilus edulis* and *Modiolus modiolus*), which were more abundant in the samples. Cultured sea scallop larvae are compared with what we have identified here as wild sea scallop larvae in Fig. 1. A complete series of micrographs of cultured animals, from straight hinge to metamorphosed larvae, can be found in Culliney (1974). For comparison with *Mytilus* and *Modiolus* larvae, refer to the micrographs in Schweinitz and Lutz (1976).

The features of the larval hinge are best illustrated by the SEM micrographs (Fig. 2). At 4 d, the hinge appeared featureless, with no teeth visible (Fig. 2b). By 7 d, several teeth were evident on the anterior and posterior sides of the hinge (Fig. 2c). This absence of teeth in the central region of the hinge is characteristic of pectinids in general (Le Pennec 1980) and is evident in the only other published micrograph of a larval *P. magellanicus* hinge (fig. 2d in Lutz et al. 1982).

The number of hinge teeth increased with increasing length of the cultured larvae. A total of 6 teeth were present on the 7-d-old larvae (Fig. 2c), while 9–10 teeth were present on the largest (Fig. 2f). The largest wild sea scallop larvae examined (~250 μm; Fig. 3) also had 9–10 hinge teeth. Because *P. magellanicus* larvae can reach almost 300 μm prior to settlement, more teeth may be present in the largest larvae.

The light micrographs of the larval hinge (Fig. 4) are less clear than those from the SEM, but are included here because for routine work, light microscopes are much more practical. Except for Fig. 4a, all are of animals caught in the Bay of Fundy in September or October 1984. In each animal, 3–5 teeth were evident on the anterior and posterior sides of the hinge.

The larvae of the few other pectinids north of Cape Cod and south of Newfoundland are not described. Although it cannot be definitively evaluated whether or not these larvae could be distinguished from *P. magellanicus* larvae, they must contribute marginally to the pool of pectinid larvae in late summer and fall. This is due to both their low abundance and possible low fecundity, which may be inferred from their small size. *Cyclopecten pustulosus*, *Palliolum striatum*, and *Delectopecten vitreus*, which occur north of Cape Cod, are all less than 2 cm in length and are relatively uncommon (Abbott 1974). Thus, even if they did spawn at the same time as *P. magellanicus* and their larvae were indistinguishable, they would be greatly outnumbered by the *P. magellanicus* larvae which are produced by a much more abundant, fecund scallop.

The only other pectinid in the area is the Iceland scallop, (*Chlamys islandica*) which, although more common than the other three pectinids, is still much less so than the sea scallop. Of 196 stations sampled by Caddy (1970) in the Bay of Fundy, *P. magellanicus* was "present" at 167, "abundant" at 100, and "common" at 20. In contrast, *C. islandica* was present at 73 stations, was common at 11, and abundant at only 2 stations. It is possible that in the Bay of Fundy and Gulf of Maine area, *C. islandica* also spawns earlier than the sea scallop. In Newfoundland waters, *C. islandica* spawns predominantly in the first half of the year (K. S. Naidu, Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Nfld., pers. comm.).

The ability to identify planktonic sea scallop larvae is the first requirement for addressing questions about the role of the larval phase in genetic exchange between adult concentrations of scallops, and in recruitment to the fishery.

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References

ABBOTT, R. T. 1974. American seashells. 2nd ed. Van Nostrand Reinhold Co., New York, NY.

BOURNE, N. 1964. Scallops and the offshore fishery of the Maritimes. Bull. Fish. Res. Board Can. 145: 60 p.

CADDY, J. F. 1970. Records of associated fauna in scallop dredge hauls from the Bay of Fundy. Fish. Res. Board Can. Tech. Rep. 225: 31 p.

1979. Long-term trends and evidence for production cycles in the Bay of Fundy scallop fishery. Rapp. P-V. Réun. Cons. Int. Explor. Mer 175: 97–108.

COUTURIER, C. Y. 1986. Aspects of reproduction and larval production in *Placopecten magellanicus* held in a semi-natural environment. M.Sc. thesis, Dalhousie University, Halifax, N.S. 108 p.

CULLINEY, J. L. 1974. Larval development of the giant scallop *Placopecten magellanicus* (Gmelin). Biol. Bull. 147: 321–332.

DICKIE, L. M. 1955. Fluctuations in abundance of the giant sea scallop, *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. J. Fish. Res. Board Can. 12: 797–857.

DOMINGUEZ, M., AND M. ALCARAZ. 1983. Planktonic larvae of lamellibranch molluscs from the Pontevedra Estuary, northwest Spain. Can. Transl. Fish. Aquat. Sci. 5179. (From: Invest. Pesq. 47: 345–357)

HURLEY, G. V., M. J. TREMBLAY, AND C. COUTURIER. 1987. Age estimation of sea scallop larvae (*Placopecten magellanicus*) from daily growth lines on shells. J. Northwest Atl. Fish. Sci. 7(2). (In press)

LE PENNEC, M. 1980. The larval and post-larval hinge of some families of bivalve molluscs. J. Mar. Biol. Assoc. U.K. 60: 601–617.

LUTZ, R., J. GOODSELL, M. CASTAGNA, S. CHAPMAN, C. NEWELL, H. HIDU, R. MANN, D. JABLONSKI, V. KENNEDY, S. SIDDALL, R. GOLDBERG, H. BEATTIE, C. FALMAGNE, A. CHESTNUT, AND A. PARTRIDGE. 1982. Preliminary observations on the usefulness of hinge structures for identification of bivalve larvae. J. Shellfish Res. 2: 65–70.

MACKENZIE, C. L. 1979. Biological and fisheries data on sea scallop, *Placopecten magellanicus* (Gmelin). Natl. Mar. Fish. Serv. Tech. Ser. 19. Sandy Hook Laboratory, Highlands, NJ.

MERRILL, A. S. 1961. Shell morphology in the larval and postlarval stages of the sea scallop, *Placopecten magellanicus* (Gmelin). Bull. Mus. Comp. Zool. 125(1): 1–20 + 3 plates.

SAVAGE, N. B. 1980. Monitoring of bivalve larvae continues off the New Hampshire coast. Coastal Oceanogr. Climatol. News 2: 44–45. (Available from University of Rhode Island, Center for Ocean Management Studies, Kingston, RI).

SCHWEINITZ, E. H., AND R. A. LUTZ. 1976. Larval development of the northern horse mussel, *Modiolus modiolus* (L.), including a comparison with the larvae of *Mytilus edulis* (L.) as an aid in planktonic identification. Biol. Bull. 150: 348–360.

SERCHUK, F. M., P. W. WOOD, J. A. POSGAY, AND B. E. BROWN. 1979. Assessment and status of sea scallop (*Placopecten magellanicus*) populations of the northeast coast of the United States. Proc. Natl. Shellfish. Assoc. 69: 161–191.

STAFFORD, J. 1912. On the recognition of bivalve larvae in plankton collections. Contrib. Can. Biol. 1906–1910: 221–242.

A General Model for Simulation of Stock and Fleet Dynamics in Spatially Heterogeneous Fisheries

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Hilborn, R., and C. J. Walters. 1987. A general model for simulation of stock and fleet dynamics in spatially heterogeneous fisheries. Can. J. Fish. Aquat. Sci. 44: 1366–1369.

A simple simulation model of stock and fleet dynamics is described that allows rapid exploration of the dynamics of fisheries in a spatially heterogeneous environment. The model simulates how fishermen allocate their search effort among spatial areas in relation to differential catch rates, prices, or area-specific desirabilities. The model runs in a few seconds per simulated year on an IBM microcomputer in interpreted BASIC, although both age structure and space are explicitly represented. The importance of exploring dynamic behavior of fisheries systems is discussed.

On décrit un simple modèle de simulation de la dynamique stock–flottille qui permet l'étude rapide de la dynamique des pêches dans un environnement hétérogène dans l'espace. Le modèle simule la manière dont les pêcheurs répartissent leur effort de recherche parmi des zones spatiales par rapport au taux de capture différentiel, aux prix et aux avantages particuliers d'une région. Le modèle, exécuté en quelques secondes par année simulée, passe en langage BASIC décodé sur un micro-ordinateur IBM; la structure selon l'âge et l'espace est malgré tout représentée en termes clairs. On examine aussi l'importance que porte l'exploration du comportement dynamique de systèmes halieutiques.

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Nearly all fish stocks exhibit some form of spatial structure. This may take the form of discrete and isolated stocks, as occurs in many invertebrates, or mobile stocks that simply are more dense in some areas than others. How fish are distributed and how fishermen allocate their fishing effort in space are essential ingredients in understanding how a fishery develops and the relationship between catch rate and abundance (R. Hilborn and C. J. Walters, unpubl. data). Clark (1982) has examined the spatial distribution of stocks using the concept of "concentration profiles," a term originally developed in mining. He considers the fact that the distribution of densities will largely determine the relationship between catch rates and abundance. MacCall (1984) has looked at a general model of spatial distribution and considered a number of implications for fisheries management, particularly in cases where the range of the stock rather than the density changes with changing abundance. Caddy (1975) has simulated the distribution of fishing effort in the Georges Bank scallop fishery. Allen and McGlade (1986) have simulated spatial patterns of fishing effort on the Scotian shelf in relation to fish stock dynamics and heterogeneity of behavior among fishermen in selecting fishing areas.

With the above exceptions, there has been remarkably little theoretical work on spatial distributions, and particularly how fishermen allocate their effort in relation to spatial distribution. Hilborn and Ledbetter (1979) showed that fishermen will move to areas where catch rates are higher, and will generally shift their effort as catch rates change. They also showed that factors such as distance from home port may override differential catch rates in fishermen's choice. Mangel and Clark (1983) have developed models for effort allocation among fishing areas, when the fish are schooling and the fishermen must learn about the abundance pattern over time (through exploratory fishing). However, only Allen and McGlade (1986) have really explored dynamic models of fishermen responding to changing spatial patterns of abundance associated with natural stock variation and fishing. One way to approach this problem is via simulation.

Simulation modelling is one of the most powerful tools available in fisheries science. The development of simulations forces explicit statements of assumptions and hypotheses, and encourages exploration of alternative hypotheses. Even very simple simulations can help to better appreciate how fish stocks will respond to exploitation. For example, anyone who has ever simulated an initially unfished population undergoing increasing exploitation rates will be quite skeptical of any stock assessment technique that assumed the stock was at equilibrium. Some surprising relationships have been discovered

almost by accident during simulation exercises. For examples, Ricker (1958, 1973) identified transient effects of fishing policies for mixed stocks using simulation, and Walters and Ludwig (1981) and Walters (1985) discovered the strongly biasing effect of measurement error and time series correlation on stock and recruitment assessments simply by simulating populations and sampling data from them.

The opportunities in fisheries for ever knowing the "true" answer about real populations are quite limited, and it is often critical to have simulations as "known systems" against which to test methods. Southward (1968), Hilborn (1979), Smith and Walters (1981), and Koonce and Schuter (1987) all used simulated populations to test specific stock assessment and management tools. As a general rule, no stock assessment technique should ever be used until the biases are carefully examined by realistic simulation of data including all types of random variability in dynamics and observation. Unfortunately, this is still not common practice, and most papers presenting a new assessment method simply show the answer for some data set, while the correct answer remains unknown.

Finally, simulation is a very powerful tool for teaching and training of fisheries managers. Paulik (1972), Schreck and Everhart (1973), and Hilborn and Walters (1986) all provide examples of simulations used for training purposes.

There remains something of a mystique about model building in fisheries, a mystique that says model building is both difficult and time consuming. We believe that most models should require a few hours to build, rather than days or months. Models that are so complicated that it takes a long time to build them will almost certainly be so complex that data are unobtainable, and will be quite difficult to understand.

Although models of single-species stock dynamics are quite commonly used and available, models that incorporate several stocks or species, and include the dynamics of fishing fleets, are quite rare. The purpose of this paper is to describe a very simple simulation model for multispecies or multistock fisheries that are spatially distributed, with realistic dynamics of a fishing fleet that moves between alternative fishing areas. We have used this model for several different fisheries, including a multispecies trawl fishery in southeastern Australia and the abalone fishery in South Australia. The model can be written in a few lines of BASIC and runs on any microcomputer and requires a few seconds per year of simulation; an IBM-PC diskette with the program is available from the first author.

The Model

Stock dynamics — While much may be learned from simple surplus production models as in Allen and McGlade (1986), most biologists are more comfortable with analyses that explicitly include the biological realisms of age structure, growth, and variable recruitment that occurs with some time lag. Although full age-structured simulations are simple to build (Walters 1969), they consume considerable computing time, which is a serious hindrance to the benefits from simulation. Fortunately, Deriso (1980) and Schnute (1985) have found an excellent computation framework for such models, fully incorporating age structure and growth and providing for alternative recruitment models. The Deriso–Schnute equations require only one program statement for biomass update, one statement for calculation of recruitment, and a loop to update age classes that have not recruited to the fishery. Their model is thus only marginally more complex than a surplus production model.

The basic Deriso–Schnute model updates biomass with the following equation:

$$(1)\quad B_{t+1} = (1 + r)S_tB_t - rS_tS_{t-1}B_{t-1} + w_kR_t - rS_tw_1R_{t-1}$$

where B_{t+1} is the biomass next year, r is the Brody growth coefficient, S_t is the total survival rate (taking account of both fishing and natural mortality) this year, B_t is the biomass this year, S_{t-1} is the total survival rate last year, B_{t-1} is the biomass last year, w_k is the weight of an individual fish at recruitment, R_t is the recruitment this year in numbers of fish, and w_1 is the weight of a fish the year before recruitment. r is the slope of the Walford plot of body weight at age $a + 1$ against weight at age a. In standard fisheries jargon using instantaneous rates, $S = \exp\{-F-M\}$.

The recruitment needs to be calculated in a separate instruction, and can be any function of stock biomass (or random and independent of biomass for skeptics of stock and recruitment relations). If we use the Beverton–Holt stock–recruitment model, the recruitment equation becomes

$$(2)\quad R_t = (B_t a)/(b + B_t)$$

where a is the maximum number of recruits possible and b is the biomass level at which recruitment is $a/2$. Here it is to be understood that the recruits R_t produced by B_t will enter the stock after a lag of 1 yr (longer delays are easily simulated).

Equations (1) and (2) can be applied to spatially isolated stocks, with each stock assigned distinctive parameter values so that it is not even critical that the stocks be of the same species. However, the modeling problem becomes considerably more complex when there may be spatial movement (dispersal, migration) among the areas. In that case the modeller must confront two basic questions: (1) at what life stage(s) does movement take place?, and (2) are movement rates density dependent? The first question determines whether movement rules need to be included just for recruits R, or for B as well. The second question is more difficult: density-dependent movement can mean either that (a) the range of the stock expands with stock size, rather than density increasing within a fixed range (MacCall 1984), or else that (b) dispersal acts to even out density variations among areas. Density-independent movements can be simulated simply by applying spatial transition matrices P_r and P_b to the vectors of area-specific recruitments R^* and B^* prior to movement, so as to give the postmovement vectors as

$$(3)\quad R_t = P_rR^* \text{ and } B_t = P_bB^*$$

where P_{ij} is the probability of successfully moving from area j to area i in a year. Simulation of density-dependent movements requires either that the P_r and/or P_b matrices be assigned values that are dependent on R^* and/or B^*, or else that the movements be simulated by a sequential algorithm that redistributes a pool of moving animals according to rules for how each area "fills up" (so that animals moved later in each year's sequence are assigned to areas that are less filled).

Fleet dynamics — Modeling of fleet dynamics is not well developed. Therefore, we will show how to include several alternative assumptions about fishermen's behavior, although we recognize that this will be an incomplete list. The main assumption will be that each year there is a fleet with a fixed number of vessels (which may change from year to year), and these vessels have alternative places to fish. Annual effort is then distributed by the algorithm shown in Table 1. To begin with,

TABLE 1. Order of events for calculating fishing effort distribution among areas.

1. Determine proportion of total effort to be allocated to each spatial area for exploratory fishing
2. Allocate exploratory effort to determine initial catch rates
3. Break remaining total effort into subunits to be allocated sequentially (10–30 subunits recommended)
4. Allocate each effort subunit sequentially as follows:
   a. Determine which area has the highest catch rate
   b. Allocate one subunit of effort to that area
   c. Determine catch from that effort
   d. Subtract catch from abundance in the area
   e. Update catch rate in that area
   f. Return to step "a" unless all subunits allocated

we will assume that the fishermen wish to maximize the landed value of their catch, and that they have perfect information about the expected catch rates and prices in each area. Then effort will be allocated to the best fishing area first, with only exploratory effort in other areas. Then when (or if) that area is fished down (so that some other area provides better catch rates), then further effort will move to that new "best" area.

To simulate this type of behavior (Table 1), we first allocate a small amount of "exploratory" effort to each area, to determine the catch rate (in tonnes, or dollars) for that area. We then partition the total potential fishing effort for the season into units of 1/10 to 1/30 of the total, and begin to allocate each of these to the "best" area. As each unit is allocated, the catch is removed from the "best" area, the catch rate is determined, and a new determination of which area is "best" is made. This is a brute force method, and we have been surprised at how quickly such an algorithm runs, even in intepreted BASIC. At all times there is a list of catch rates obtained by the last unit of effort in each area; this list is initially determined by the exploratory fishing effort, and then updated each time additional effort is allocated. Provided the effort units are small enough to take only a small fraction of the stock in each allocation step, and provided handling times for each unit of catch are small, the catch in each area (Table 1, step 4c) for a multispecies fishery can be calculated simply as

$$(4) \quad C_{ij} = B_{ij} E_j q_i$$

where C_{ij} is the catch of the ith species in the jth area, B_{ij} is the biomass of fish of the ith species in the jth area, E_j is the effort allocated to the jth area, and q_i is the catchability of the ith species (q could be subscripted by area as well as species).

Assuming that fishermen are attempting to maximize value of catch, we would calculate the catch rate (Table 1, step 4e) as the catch times the price for the fish:

$$(5) \quad U_j = \sum_i C_{ij} p_i / E_j$$

where U_j is the dollars per unit of effort in area j and p_i is the price per fish of the ith species.

The sequential effort allocation model can be modified in various ways to make it more realistic. First it may be that not all fishermen are trying to maximize income; other factors such as distance from home and risk may be quite important (Hilborn and Ledbetter 1979; Allen and McGlade 1986). In this case, each area can be assigned a relative preference (0–1 index) that is multiplied times the catch rate (in Table 1, step 4e), and the resulting weighted index in turn used to decide which area is best. This will result in the biological catch rate (in tonnes or dollars) being higher in the less preferred areas.

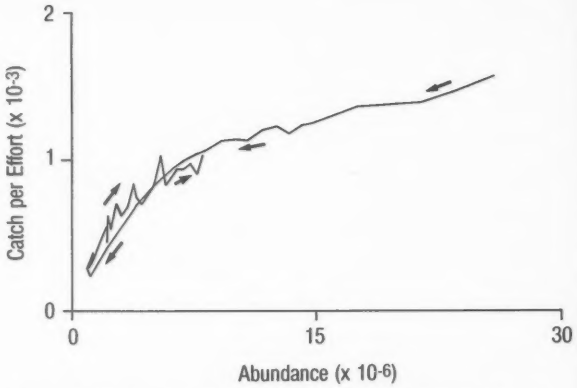

FIG. 1. Relationship between catch rate and abundance as effort increases for the abalone model described in the text. Catch rate remains high as abundance declines because of the handling time in the catch equation and the sequential movement of effort from desirable to undesirable areas.

Second, fishermen may try to maximize profit rather than landed value; this possibility can be reflected in the U index of eq. (5) by subtracting off an area-specific cost per unit of effort.

Another important possibility is that instead of the total fishing effort for a season being fixed, the total may depend upon the catch rate. When catch rates are higher, more effort will be exerted. This is easily incorporated by defining a minimum catch rate such that no more fishing effort is allocated within a year if (or after) all areas fall below this minimum.

Finally, it may well be that a portion of the fishing effort will go to each area, regardless of the catch rates. This could be due to imperfect information or individuals who will only fish specific areas. It is easily incorporated simply by modifying the amount of "exploratory" effort assigned to each area (Table 1, step 2).

Results

Figure 1 shows the results of a simulation of six discrete abalone stocks, with the fishing fleet (total effort each year) increasing for 30 yr and then being cut back to low levels. Biological parameters (growth, longevity, recruitment) for the stocks were chosen to be roughly representative of major Australian abalone fisheries (Tasmania, New South Wales, South Australia). Since there is a significant handling time h associated with the capture of each abalone by a diver, eq. (4) is not appropriate to describe the local catch rates. Instead we used a standard type II functional response (Table 1, step 4c) given by

$$(6) \quad \mathrm{CPUE}_i = (a\, v_i\, B_i) / (1 + h\, a\, v_i\, B_i)$$

where CPUE_i is the catch in kilograms per diver day in area i, a is the area searched in square metres per day, v_i is the probability that an individual abalone in area i will be found in the area searched ($a \cdot v$ is the standard catchability q), B_i is the abundance in kilograms per square metre of abalone in area i, and handling time h is measured in days per kilogram captured.

Note in Fig. 1 that the catch rate initially declines much more slowly than abundance. This is because the fishermen are sequentially fishing each area down from a virgin state, so that catch rates remain at a high level until the fishery is reasonably fully developed on all sites, and then the catch rate starts to drop rapidly once the least desirable virgin stock is fished down.

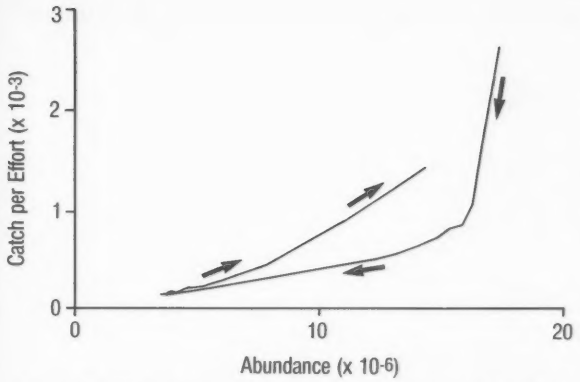

FIG. 2. Relationship between catch rate and abundance when handling time is reduced and one area is given a very high initial catch rate.

TABLE 2. Components that can be included in model.

Biological elements	Fleet elements
Age structure	Effort goes where fishing best
Growth	Differential desirability by area
Stock–recruitment	
Spatially discrete stocks	Exploratory effort to each area
Stock redistribution each year to each area	Minimum acceptable catch rate
Interspecific competition	Effort responds to catch rate
Predation	Area-specific costs and prices
	Several fleets

Figure 2 shows a modification of the above model, in which one area was assigned a much higher initial biomass than the other areas, and lower total area. In this case the initial catch rates are very high but drop quickly as that one high abundance area is fished down. Total biomass over all areas is little affected during early development, since most effort is attracted to the one best area. Also note that the recovery path from the low abundance point is not along the same trajectory as the descent; this effect would not be predicted by simpler models of effort distribution and catch rate, which would ignore the selective "focussing" of effort on the high density area as it recovers while total effort remains low.

Discussion

The model described above incorporates a wide variety of biological and social mechanisms (Table 2). In addition to factors included in the sample results of Fig. 1 and 2, many other mechanisms can be easily added. On the ecological side, interspecific competition and/or predation effects could be added to the recruitment term of the Deriso–Schnute equations, and exchange by movement between areas could be added to provide a homogenizing effect from year to year.

Additional economic detail can also be added to the fishing effort dynamics, including area-specific costs, area-specific prices, and price decreasing with volume of catch. Several discrete fleets or types of vessels could also be added, although these would increase computational time accordingly.

The model can be used with slight modification for a wide variety of biological and economic situations. We have used it primarily to gain more understanding about the behavior of catch per unit of effort in relation to abundance for fisheries with spatially structured fishing effort. Conversion to other fisheries should require only a few hours (the original program required 3 h to design, code, and debug), so workers should not hesitate to experiment with a wide variety of assumptions and dynamics.

Acknowledgements

The model we describe was built as part of a series of workshops sponsored by the Australian Government through the Fishing Industry Research Trust Account. We especially thank the Australian scientists who work on the South East Trawl and various abalone fisheries for many of the ideas we incorporated in this model.

References

ALLEN, P. M., AND J. M. MCGLADE. 1986. Dynamics of discovery and exploitation: the case of the scotian shelf groundfish fisheries. Can. J. Fish. Aquat. Sci. 43: 1187–1200.

CADDY, J. F. 1975. Spatial model for an exploited shellfish population, and its application to the Georges Bank scallop fishery. J. Fish. Res. Board Can. 32: 1305–1328.

CLARK, C. W. 1982. Concentration profiles and the production and management of marine fisheries, p. 97–112. *In* W. Eichhorn [ed.] Economic theory of natural resources. Physica-Verlag, Wurzburg-Wien.

DERISO, R. B. 1980. Harvesting strategies and parameter estimation for an age-structured model. Can. J. Fish. Aquat. Sci. 37: 268–282.

HILBORN, R. 1979. Comparison of fisheries control systems that utilize catch and effort data. J. Fish. Res. Board Can. 36: 1477–1489.

HILBORN, R., AND M. LEDBETTER. 1979. Analysis of the British Columbia salmon purse seine fleet: dynamics of movement. J. Fish. Res. Board Can. 36: 384–391.

HILBORN, R., AND C. J. WALTERS. 1986. Microcomputer fisheries simulations as training and teaching tools. Env. Software 1: 156–163.

KOONCE, J., AND B. J. SHUTER. 1987. Influence of various sources of error and community interactions on quota management of fish stocks. Can. J. Fish. Aquat. Sci. 44(Suppl. 2). (In press)

MCCALL, A. D. 1984. Population models of habitat selection, with application to the northern anchovy. Natl. Mar. Fish. Serv. Southwest Fish. Cent. Admin. Rep. No. LJ-84-01: 98 p.

MANGEL, M., AND C. W. CLARK. 1983. Uncertainty, search, and information in fisheries. J. Cons. Perm. Int. Explor. Mer 48: 93–103.

PAULIK, G. J. 1972. Digital simulation modeling in resource management and the training of applied ecologists, p. 373–418. *In* Systems analysis and simulation in ecology. Vol. II. Academic Press Inc., New York, NY.

RICKER, W. E. 1958. Maximum sustained yields from fluctuating environments and mixed stocks. J. Fish. Res. Board Can. 15: 991–1006.

1973. Two mechanisms that make it impossible to maintain peak-period yields from stocks of Pacific salmon and other fishes. J. Fish. Res. Board Can. 30: 1275–1286.

SCHNUTE, J. 1985. A general theory for analysis of catch and effort data. Can. J. Fish. Aquat. Sci. 42: 414–429.

SCHRECK, C. B., AND W. H. EVERHART. 1973. Computer teaching exercises in fisheries science. Trans. Am. Fish. Soc. 102: 656–659.

SMITH, A. D. M., AND C. J. WALTERS. 1981. Adaptive management of stock–recruitment systems. Can. J. Fish. Aquat. Sci. 38: 690–703.

SOUTHWARD, G. M. 1968. A simulation of management strategies in the Pacific Halibut fishery. Int. Pac. Halibut Comm. Sci. Rep. 47: 70 p.

WALTERS, C. J. 1969. A generalized computer simulation model for fish population studies. Trans. Am. Fish. Soc. 98: 505–512.

1985. Bias in the estimation of functional relationships from time series data. Can. J. Fish. Aquat. Sci. 42: 147–149.

WALTERS, C. J., AND D. LUDWIG. 1981. Effects of measurement errors on the assessment of stock–recruitment relationships. Can. J. Fish. Aquat. Sci. 38: 704–710.

On Managing Variable Marine Fisheries

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Marine fisheries are difficult to manage because they are highly variable; yet the stock must be protected from over exploitation and possible collapse. Here we demonstrate that the most important source of uncertainty in estimating long-term productivity of a fishery is the degree of density dependence in the stock–recruitment relationship and not the environmental factors that influence annual recruitment rates. We evaluate the performance of three harvest policies in managing dynamically contrasting fisheries and conclude that close to maximum expected sustainable yield can be obtained by policies that have very different implications for short-term yields. In stochastic fisheries, the variability inherent in the stock can be ignored in determining optimal effort levels or it can be transferred into the fishery by allowing yield and effort levels to vary considerably. Thus it is relatively easy for the manager to ensure long-term maximization of yield. The hard problem is finding a suitable compromise between short-term stability of the fishery and long-term viability of the stock.

La forte variabilité des stocks marins rend ceux-ci difficiles à gérer, bien qu'il soit nécessaire de les protéger de la surexploitation et d'un éventuel effondrement. Les auteurs montrent que la plus importante source d'incertitude de l'estimation de la productivité à long terme d'une pêche a trait à la dépendance envers la densité de la relation stock–recrutement et non aux facteurs environnementaux influant sur les taux de recrutement annuels. Ils évaluent le rendement de trois politiques de récolte appliquées à la gestion de pêches de dynamiques très différentes et concluent qu'il est possible d'obtenir un rendement soutenu approchant la valeur maximale prévue en appliquant des politiques qui peuvent avoir des effets très différents sur les rendements à court terme. Dans le cas des pêches stochastiques, la variabilité inhérente au stock peut être ignorée lors de la détermination des niveaux d'effort optimaux, ou intégrée à la pêche si l'on autorise une forte variation du rendement et de l'effort. Il est donc relativement facile pour le gestionnaire d'obtenir une maximisation à long terme du rendement. Le problème le plus difficile à résoudre a trait à la détermination d'un compromis acceptable entre la stabilité à court terme de la pêche et la viabilité à long terme du stock.

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The primary goal of agencies that regulate the operation of marine fisheries is to ensure the continuing productivity of the resource. Beyond this, regulations can be devised to improve the efficiency of exploitation, especially in highly valuable fisheries where competition between fishermen leads to biological overfishing (Gordon 1954; Clark 1985). Regulations are typically designed to limit the annual catch to a particular value referred to as the catch "quota." Determining an appropriate set of regulations is itself a problem. Here we will only be concerned with the question of determining the quota as a function of the present state of the resource (fish stock).

It is extremely difficult to determine a quota that is considered reasonable by all parties interested in the exploitation of a particular stock. Not only does each fisherman operate under a different set of economic constraints, but parties at the marketing level and sometimes the environmental conservation level are also concerned with the magnitude of the quota. Further, there is a great deal of uncertainty associated with estimating the current state of the stock, specifically the number of individual fish by age and/or the biomass of the stock. Finally, once this estimate has been made, it must be compared with some standard value to determine whether the stock is under- or over-exploited. Usually, a reference point is obtained by estimating stock levels that correspond to the maximum average long-term yield that can be sustained under judicious harvesting practices. This maximum expected sustainable yield (MESY) is an idealized notion. It is limited by changes in productivity that may occur in fish stocks over the long term, especially in response to harvesting pressure. Also, as we demonstrate below, estimating MESY and its associated stock level is dependent on the models we select and on the quality of the data used in the estimation process.

The environment that impinges on any fish population is

stochastic, making it impossible to predict precise changes in the stock from year to year. More important, however, is the fact that larval survival rates are so critically dependent on environmental factors that future recruitment (youngest year class of fish that are vulnerable to fishing) is uncorrelated with moderate or good stock levels (at very low stock levels, future recruitment is limited by the number of eggs that are spawned). Although most of the theory relating to sustained yield fisheries management ignores stochastic effects (for example, yield-per-recruit analysis or surplus production models), the essence of the fisheries management problem is making decisions in the face of uncertainty. In particular, good management should be able to take advantage of higher than average biomass in the stock as one or more strong year classes pass through the fishery or to protect the stock when biomass is low.

Recently, three contrasting methods were investigated for setting quotas under deterministic nonequilibrium conditions in the South African anchovy (*Engraulis capensis*) fishery (Getz 1985). The policy that maximized yield over a 10-yr period yielded only 1% more biomass than either the best constant (over the 10-yr period) fishing effort policy or best constant escapement policy. The latter, which from hereon we will more accurately refer to as a base stock policy, closes the fishery whenever the stock biomass falls below a predetermined level and otherwise allows the catch to reduce stock biomass to this level. Clearly, this policy minimizes annual variation in the state of the stock, while the constant effort policy produces much less variation in the annual catch. Thus, achieving close to maximum yield is easy under a variety of management policies. The question of whether the variability should be pushed into the catch or stock is harder to address. The answer depends on economic issues as well as our perception of how vulnerable the stock is to collapse when exploited at low stock levels. In a stochastic fishery, recruitment will always be perturbed from equilibrium conditions. Here we investigate how well these "close to optimal" policies perform in three stochastic fisheries with varying dynamic characteristics. Our approach parallels the work of Ruppert et al. (1985) but uses a different stock–recruitment function.

Model

The model we use includes age structure and a nonlinear stock–recruitment relationship. Specifically, the number of individuals in each age class is reduced through time by a natural mortality rate M and a fishing mortality rate $F = qv$, where q is an age-specific catchability coefficient and v is a harvesting effort (fishing intensity) variable. That is, if $N_i(t)$ is the number of fish in age class i at time t, then

$$(1)\quad N_{i+1}(t+1) = N_i(t)e^{-(M+q_iv)}, \qquad i = 1, \ldots, n-1.$$

If w_i is the average weight of an individual in age class i, then the stock biomass B at time t is

$$(2)\quad B(t) = \sum_{i=1}^{n} w_iN_i(t).$$

If the parameter R_0 is used to denote the mean level of recruitment corresponding to the unexploited stock biomass denoted by B_0, and r is the age at recruitment, then the expected level of recruitment is given by the relationship

$$(3)\quad N_1(t+r) = R_0(B(t)/B_0)^{\rho} \qquad 0 < \rho < 1$$

where ρ is a shape parameter that determines how rapidly recruitment levels off as the stock increases. Note that when $\rho = 0$, recruitment is independent of stock biomass, and when $\rho = 1$, recruitment is proportional to stock biomass. Relationship (3) does not include the case of overcompensation in which total recruitment actually decreases per unit increase in stock biomass when the biomass is at very high levels. Overcompensation is thought to occur in the case of salmon when spawning adults feed on the fry that hatched during the previous cycle. This phenomenon was first described by Ricker (1950) for the Fraser River sockeye salmon (*Oncorhynchus nerka*).

Various techniques are available that use catch-at-age and effort data to estimate the natural mortality rates and catchability coefficients as well as the variables $N_i(t)$ in equation (1) (Pope 1972; also see Gulland 1983). Then for several values of t the estimates $N_1(t+r)$ and $B(t)$ can be paired to obtain estimates of the parameters B_0, R_0, and ρ. For the simulation studies and comparative analysis between fisheries presented below, we are interested in the effect that varying the value of ρ has on the estimates of productivity, rather than finding the best estimate of ρ itself. Thus we estimated R_0 as the value of recruitment necessary to produce a standardized unexploited biomass B_0 and expressed such quantities as yield and stock as a percentage of B_0.

We investigated the effects of stochastic environmental perturbations to recruitment on various harvesting strategies by introducing a random variable into equation (3). Specifically, we multiplied the right-hand side of (3) by $X = e^{(1+\sigma Z)}$, where Z is a Gaussian random variable drawn from the standard normal distribution ($N(0,1)$). For this definition of X, it is easily shown that its mean value is 1 and its coefficient of variation in $100\sigma\%$. Since the mean value of X is constant for all σ, any observed changes in long-term yield and stock levels as a function of σ will be due to variation in recruitment rather than changes in the expected level of recruitment (also see Hightower and Grossman 1985; and references therein).

Analysis

Uncertainty in harvesting and managing marine fisheries has been considered in several contexts. Using a Ricker surplus yield production model, Beddington and May (1977) showed that the coefficient of variation associated with the yield increases with increasing levels of exploitation, while Reed (1979) has shown than an MESY base stock policy is optimal for fairly general discrete time surplus yield models. In other studies, Aron (1979) compared the performance of constant effort, base stock, and policies intermediate between these, while Walters (1975) considered the trade-off between yield and variance. Because the market value and fecundity of individual fish are usually directly related to size, and because concepts such as yield-per-recruit depend on assessing growth and catchability as a function of age, it is often important to consider age structure in managing marine fisheries. This has been done both in the context of estimating the variances associated with the stock and yield variables during harvesting (Horwood 1982, 1983; Horwood and Shepherd 1983; Reed 1983; Getz 1984) and in the context of designing close-to-optimal management policies (Mendelssohn 1978; Lovejoy 1986; Ruppert et al. 1985). In the context of general surplus yield models, May et al. (1978) concluded that "robustly self-correcting strategies" are needed to harvest populations in uncertain environments. This is equally true for age-structured populations.

TABLE 1. Population parameters for three fisheries.

Parameter	Stock		
	South African anchovy	Bering Sea pollock	Pacific ocean perch
Number of age classes in model (n)	5	9	13
Age at recruitment to fishery (r yr)	1	2	6
Natural mortality rate (M) (yr^{-1})	0.8	0.4	0.05
Weight (g) at:			
recruitment (age r)	1.6	160	454
final age (age $n + r$)	24	1200	1360
Recruitment level (R_0)[a]	131 750	936	45

[a]R_0 is measured in numbers per metric ton of B_0 (see text for more details).

Here we address questions relating to the performance of self-correcting base stock policies and constant effort harvesting policies when harvesting marine fish populations in a stochastic environment. We also investigate how estimates of a fishery's productivity change as the assumed or fitted shape of the stock–recruitment relationship (that is, value of the parameter ρ) changes. The analysis is carried out for the following three fisheries: the fishery on South African anchovy, a short-lived, relatively fast growing species; the fishery on the United States – Canada West Coast for Pacific ocean perch (*Sebastes alutus*), a long-lived, slow growing species; and the fishery on United States Bering Sea walleye pollock (*Theragra chalcogramma*), a species that is intermediate in growth and survival with respect to the other two species. The population parameters for these fisheries are given in Table 1.

In many fisheries, especially those that have not yet developed to high levels of exploitation, stock–recruitment data are unavailable at low stock densities. This makes it almost impossible to obtain reliable direct estimates of the shape parameter ρ. We investigated how the value of ρ affects estimates of the productivity of the stock by carrying out deterministic maximum sustainable yield (MSY) analyses (after Getz 1980) in all three fisheries for the following values of ρ: 0.00, 0.25, 0.50, 0.75, and 0.95 (Fig. 1). The constant recruitment case $\rho = 0$ is equivalent to Beverton and Holt's (1957) yield-per-recruit analysis. The linear case $\rho = 1$ is equivalent to harvesting a Leslie (1945) matrix model, but, being linear, has no nontrivial equilibrium (a nonzero yield level cannot be sustained for any effort values). Thus the stock biomass curves in Fig. 2 apply only for $\rho < 1.0$. For most stocks, one would expect $\rho < 0.5$ (for example see Kimura et al. 1984), but it is clear from Fig. 2 that, even for these values of ρ, MSY as a percentage of the unexploited biomass level B_0 is quite sensitive to the actual value of ρ. As ρ ranges between 0.0 and 0.5, MSY/B_0 ranges between 0.1 and 0.2 for anchovy, between 0.05 and 0.15 for pollock, and between 0.01 and 0.03 for Pacific ocean perch. Thus we not only have a two- or three-fold decrease in MSY as a proportion of B_0 as ρ ranges over [0.0, 0.5], but Gullands rule of thumb — that MSY is approximately given by $MB_0/2$ (recall that M is the natural mortality rate) — overestimates MSY as a function of B_0 by up to 100% for the anchovy fishery. This bears out Beddington and Cooke (1983) (also see Clark et al. 1985) who concluded that Gulland's rule overestimates long-term productivity.

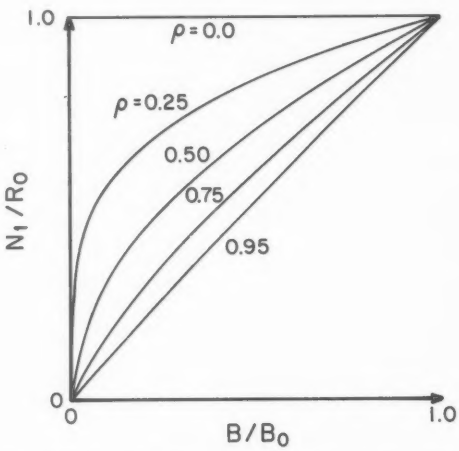

FIG. 1. The stock–recruitment curve defined in equation (3) is illustrated for different values of the shape parameter ρ used to obtain the results depicted in Fig. 2.

MESY values for the three fisheries discussed here were obtained by averaging yield over a number of different 1000-yr Monte Carlo simulation runs, where each run corresponded to a different constant level of effort, and effort values were steadily increased from 0 until a maximum of these average yields was obtained (that is, the average yields started to decrease with increased effort). First we found that the MESY in all fisheries decreases as the coefficient of variation (CV) of recruitment increases from 0 to 200%. The effect is negligible for Pacific ocean perch, but is more in evidence for short-lived species such as anchovy, where the stochastic effects of varying year class strength have a greater impart on the overall stock biomass levels available to the fishery each year. When compared with the results obtained for the different values of ρ, the amount of environmental variability is much less critical than a good estimate for ρ in assessing long-term productivity. Thus for anchovy, the most environmentally sensitive of the three stocks, a CV of 100% results in a 3% reduction in MESY compared with MSY (Fig. 3), but the MSY itself changes by more than 50% when ρ increases from 0.0 to 0.5 (Fig. 2A). Note that Clark (1985) considered variability to be "low" when

TABLE 2. Average yield, effort, and stock biomass levels, with associated coefficients of variation in parentheses, obtained from an MESY constant effort policy (CE), an MESY base stock policy (BS), and an exploitative policy (EX) evaluated over 1000-yr computer runs using Monte Carlo simulation techniques (see text for more details).

Stock	Policy	Yield[a] (%)	Effort[b]	Stock[a] (%)	Minimum effort[c] (%)	Biomass below $B_{MESY}/2$ (%)[d]
Anchovy	CE	15.5 (52)	1.2 (0%)	16.0 (57)	0	14
	BS	14.0 (92)	1.1 (83%)	15.9 (2)	5	0
	EX	14.5 (68)	1.4 (39%)	12.0 (30)	71	16
Pollock	CE	8.8 (50)	0.52 (0%)	16.5 (49)	0	9
	BS	8.6 (103)	0.50 (95%)	16.5 (0)	2	0
	EX	8.7 (75)	0.63 (49%)	12.4 (26)	79	12
Pacific ocean perch	CE	1.7 (22)	0.12 (0%)	22.5 (22)	0	0
	BS	1.7 (82)	0.12 (88%)	22.5 (0)	0	0
	EX	1.7 (51)	0.14 (50%)	19.7 (14)	84	0

[a]Yield and stock biomass levels are expressed as a percentage of the virgin stock biomass B_0.
[b]Units for measuring effort are scaled within the model by the catchability coefficients and in this table are only useful for within-fishery policy comparisons.
[c]Percentage of years that minimum effort is employed. In the case of the BS policy, minimum effort is zero; that is, the entry is the percentage of years that the fishery is closed.
[d]Measured in percentage of years.

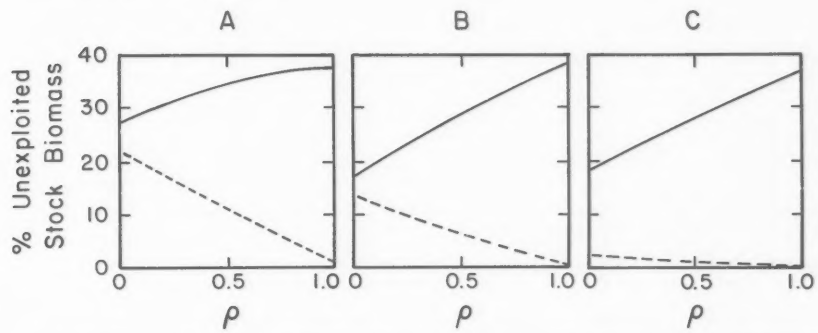

FIG. 2. Maximum sustainable yield (broken line) and corresponding stock biomass levels (solid line), expressed as a percentage of the unexploited stock biomass B_0, are plotted as a function of the stock–recruitment shape parameter ρ (see equation (3)) for the (A) South African anchovy, (B) Bering Sea pollock, and (C) Pacific ocean perch fisheries.

the CV is less than 50% and to be "high" when the CV is greater than 100%. Also, in marine fisheries of the type considered here, CV's in the region of 10–120% have been observed (Hightower and Grossman 1985).

The problem of harvesting stocks that exhibit considerable recruitment variability has been investigated in the case of Atlantic menhaden (*Brevoortia tyrannus*) by Ruppert et al. (1985) and in the cases of South African anchovy, Atlantic menhaden, and Pacific ocean perch by Hightower and Grossman (1985). The latter authors considered the response of yields and associated variances under constant harvesting policies corresponding to MESY levels (that is, v, $= v_{MESY}$) and to 1.25, 1.50, 1.75, and 2.00 times v_{MESY}. They concluded that it is difficult to assess differences in yield when environmental variability is relatively high (CV = 75%) and $v < 1.5v_{MESY}$. In Table 2 we present results pertaining to harvesting three stocks under three contrasting harvest policies. These results were obtained from 1000-yr Monte Carlo simulation runs where the CV was 100% and the stock–recruitment shape parameter ρ had a value of 0.25 for all fisheries and in all runs. The three policies were as follows: (1) $v = v_{MESY}$ each year (MESY constant effort policy); (2) v was selected so that the stock biomass at the end of the year was B_{MESY}, or if the stock was particularly weak and this was not possible, then $v = 0$ (MESY base stock policy); (3) v was selected to be the maximum of v_{MESY} and the value of V that would result in a stock biomass of B_{MESY} at the end of the year (exploitative policy). The third policy is referred to as exploitative because it increases the fishing effort beyond B_{MESY} when the stock would otherwise be above B_{MESY}, but does not reduce v below v_{MESY} when the resulting stock is below B_{MESY}.

The results in Table 2 indicate that long-term yields are very similar under all policies, with the greatest deviation occurring in the case of anchovy where the base stock policy results in 7% less yield when compared with the constant effort policy. This contrasts with Ruppert et al. (1985) who found that the best base stock policy for Atlantic menhaden produced 17% higher long-term average yields than the best constant effort policy. Although our MESY base stock policy is not necessarily the base stock policy that maximizes expected sustainable yields,

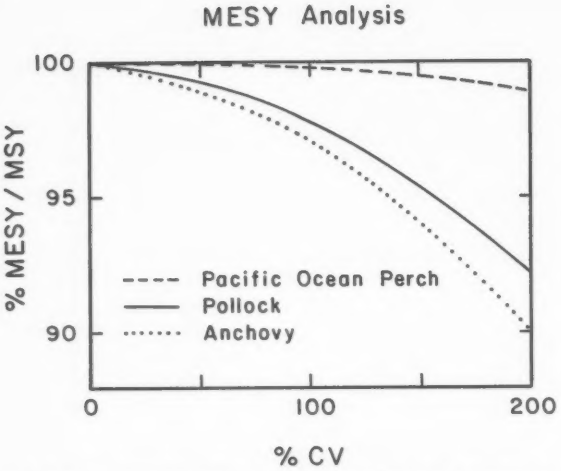

FIG. 3. Maximum expected sustainable yield (MESY) as a percentage of maximum sustainable yield (MSY) is plotted as a function of increasing levels of stochasticity in the stock–recruitment relationship. The independent variable %CV represents the coefficient of variation associated with a lognormally distributed random variable (mean = 1) that enters multiplicatively into equation (3) (see text for details).

the strikingly large disparity in the constant effort and base stock policies of Ruppert et al. (1985) may be in error (see Hightower and Lenarz 1987). For stocks without age structure, Reed (1979) has shown that the MESY base stock policy is optimal (also see Clark 1985). For age-structured stocks the question of which base stock policy is optimal remains open.

Discussion

Our study suggests that as long as a harvesting policy is designed around MESY conditions, long-term yield is relatively unaffected by the policy in question. The socioeconomic implications of the various policies are vastly different, however, as are the associated risks. For example, the base stock policy closes the fishery down, or at least substantially reduces yield when stock biomass is low. In the case of the anchovy, the base stock policy closed the fishery on average once every 20 yr. Such closures may protect the stock from a possible collapse, but the high variability in annual catch and hence income for participants in the fishery could be economically disasterous. The constant effort policy, on the other hand, reduces the variance in annual catch by pushing more variance into the stock biomass levels. This may be satisfactory, provided the stock is afforded some measure of protection when biomass levels are low. The exploitative policy may be the easiest to justify politically, since the fisherman are allowed to take advantage of the stock when it is strong and are otherwise allowed to fish at MESY effort levels. However, the average stock biomass levels were reduced by 10–25% when compared with the other two policies so that the risk of the fishery collapsing would likewise be increased. For comparative purposes we selected $B_{MESY}/2$ as an indicator of poor stock condition. From the last column in Table 2 we see that on average the pollock population drops below $B_{MESY}/2$ every eleventh year under an MESY constant effort policy and approximately every eighth year under an exploitative policy.

In summary, our results suggest that uncertainty in estimating MESY is more influenced by the shape of the stock–recruitment relationship, especially the rate at which recruitment falls off at low stock levels, than it is on the level of environmental variability associated with the recruitment process. Further, since fundamentally different policies are often indistinguishable on the basis of yield, the "best" policy must be determined by balancing yield and effort variability against stock variability and the probable risk of the fishery collapsing. Thus, setting quotas is primarily a risk assessment and fisheries stability problem rather than a yield maximumization problem.

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References

ARON, J. L. 1979. Harvesting a protected population in an uncertain environment. Math. Biosci. 47: 197–205.

BEDDINGTON, J. R., AND J. COOKE. 1983. The potential yield of previously unexploited stocks. FAO Fish. Tech. Pap. No. 242. Food and Agriculture Organization of the United Nations, Rome.

BEDDINGTON, J. R., AND R. M. MAY. 1977. Harvesting natural populations in a randomly fluctuating environment. Science (Wash., DC) 197: 463–465.

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. U.K. Minist. Agric. Fish. Food (Lond.) Fish. Invest. (Ser. 2) 19: 533 p.

CLARK, C. W. 1985. Bioeconomic modelling and fisheries management. Wiley-Interscience, New York, NY.

CLARK, C. W., A. T. CHARLES, J. R. BEDDINGTON, AND M. MANGEL. 1985. Optimal capacity decisions in a developing fishery. Mar. Resour. Econ. 2: 25–53.

GETZ, W. M., 1980. Harvesting models and stock–recruitment curves in fisheries management. *In* W. M. Getz [ed.] Mathematical modelling in biology and ecology. Springer Verlag, Heidelberg.

1984. Production models for nonlinear stochastic age-structured fisheries. Math. Biosci. 69: 11–30.

1985. Optimal and feedback strategies for managing multicohort populations. J. Optim. Theor. Appl. 46: 505–514.

GORDON, H. S. 1954. The economic theory of a common properyty resource: the fishery. J. Polit. Econ. 62: 124–142.

GULLAND, J. A. 1983. Fish stock assessment: a manual of basic methods. John Wiley & Sons, New York, NY.

HIGHTOWER, J. E., AND G. D. GROSSMAN. 1985. Comparison of constant effort harvest policies for fish stocks with variable recruitment. Can. J. Fish. Aquat. Sci. 42: 982–988.

HIGHTOWER, J. E., AND W. H. LENARZ. 1987. Optimal harvesting policies for the widow rockfish fishery. *In* E. A. Vetter and B. E. Megrey [ed.] Mathematical analysis of stock dynamics. (In press)

HORWOOD, J. W. 1982. The variance of population and yield from an age-structured stock, with application to North Sea herring. J. Cons. Int. Explor. Mer 40: 237–244.

1983. A general linear theory for the variance of yield from fish stocks. Math. Biosci. 64: 203–225.

HORWOOD, J. W., AND J. G. SHEPHERD. 1983. The sensitivity of age-structured populations to environmental variability. Math. Biosci. 57: 59–82.

KIMURA, D. K., J. W. BALSIGER, AND D. H. ITO. 1984. Generalized Stock Reduction Analysis. Can. J. Fish. Aquat. Sci. 41: 1325–1333.

LESLIE, P. H. 1945. On the use of matrices in certain population mathematics. Biometrika 35: 183–212.

LOVEJOY, W. S. 1986. Bounds on the optimal age-at-first-capture for stochastic, age-structured fisheries. Can. J. Fish. Aquat. Sci. 41: 101–107.

MAY, R. M., J. R. BEDDINGTON, J. W. HOWROOD, AND J. G. SHEPHERD.

1978. Exploiting natural populations in an uncertain world. Math. Biosci. 42: 219–252.

MENDELSSOHN, R. 1978. Optimal harvesting strategies for stochastic single species, multiage class models. Math. Biosci. 41: 159–174.

POPE, J. G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Res. Bull. ICNAF 9: 65–74.

REED, W. J. 1979. Optimal escapement levels in stochastic and deterministic harvesting models. J. Environ. Econ. Manage. 6: 350–363.

1983. Recruitment variability and age-structure in harvested populations. Math. Biosci. 65: 239–268.

RICKER, W. E. 1950. Cycle dominance among the Fraser sockeye. Ecology 31: 6–26.

RUPPERT, D., R. L. REISH, R. B. DERISO, AND R. J. CARROLL. 1985. A stochastic population model for managing the Atlantic menhaden (*Brevoortia tyrannus*) fishery and assessing managerial risks. Can. J. Fish. Aquat. Sci. 42: 1371–1379.

WALTERS, C. J. 1975. Optimal harvesting strategies for salmon in relation to environmental variability and uncertain production parameters. J. Fish. Res. Board Can. 32: 1777–1784.

Influence of Three Life History Strategies on the Information Content of Stock–Recruitment Data

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Welch, D. W. 1987. Influence of three life history strategies on the information content of stock–recruitment data. Can. J. Fish. Aquat. Sci. 44: 1375–1379.

The information content of stock–recruitment (SR) data is examined for three population models incorporating different life history characteristics. These models represent (i) a population whose recruitment is totally independent of stock size, (ii) a semelparous population whose recruitment is dependent on the abundance of the adult age group, and (iii) an iteroparous population whose recruitment is equally dependent on the abundance of several age groups. Fourier analysis of these age structures shows that the recruitment data (i) are completely uninformative about SR processes at all frequencies, (ii) are equally informative about SR processes at all frequencies, and (iii) contain more information about SR processes at some frequencies than at others. In particular, the SR information content of the latter model is shown to be zero at certain frequencies, so that all such recruitment variability can be removed from the data by filtering before examining the SR relationship. A numerical example is presented for the last model.

L'auteur examine la teneur informative des données stock–recrutement (SR) dans le cas de trois modèles de population comprenant diverses caractéristiques de cycle vital. Ces modèles représentent i) une population dont le recrutement est totalement indépendant de la taille du stock, ii) une population sémelpare dont le recrutement est dépendant de l'abondance du groupe d'âge des adultes et iii) une population itéropare dont le recrutement dépend également de l'abondance de divers groupes d'âge. Une analyse de Fourier de ces structures par âges a permis de montrer que les données de recrutement i) ne fournissent aucune information quant au processus SR quelles que soient les fréquences, ii) sont aussi informatives quant au processus SR à toutes les fréquences et iii) contiennent plus d'information sur les processus SR à certaines fréquences. Plus particulièrement, l'auteur montre que la teneur informative des données SR du dernier modèle est égale à zéro à certaines fréquences de sorte que toute cette variabilité quant au recrutement peut être soustraite des données par filtrage avant l'étude de la relation SR. Un exemple numérique est donné pour le dernier modèle.

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A fundamental problem in population ecology has been the identification of density-dependent processes regulating the abundance of natural populations. The difficulties involved in such studies are particularly critical in fisheries science, since the response of populations to exploitation depends on both the form and the strength of any density-dependent regulation. However, the ability to resolve such factors is complicated by the need to distinguish between environmental and density-dependent influences on recruitment, as well as errors introduced by estimating recruitment strengths from catch data.

The identification of the density-dependent mechanisms determining the stock–recruitment relationship (SRR) for iteroparous populations can be simplified when stock is a function of the density of two or more age groups. In such cases, Fourier analysis may be employed to show the fraction of the total recruitment variability present at different frequencies which can be associated with stock effects (Welch 1986).

The relative strength of the density-dependent processes affecting recruitment can therefore be increased by filtering out the recruitment variability unrelated to variations in stock size. This makes the identification of the SRR more precise, a result previously demonstrated by Monte Carlo analysis (Welch 1987a). The purpose of this note is to analyze the contribution of filtering theory to stock–recruitment (SR) analysis for three simple population models which represent the extremes of

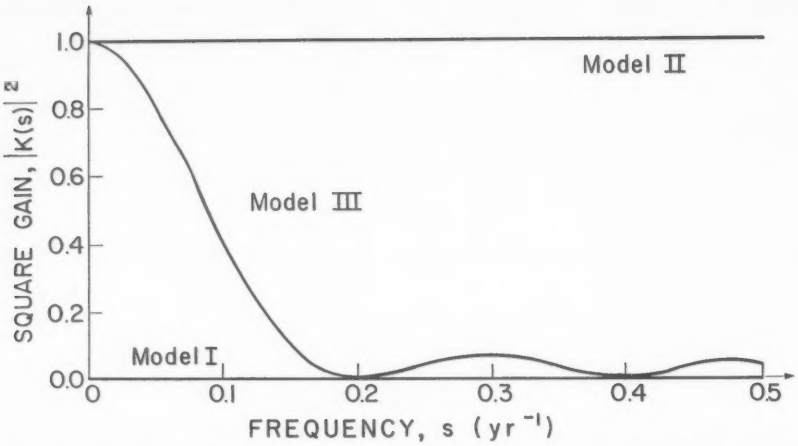

FIG. 1. Squared gain for the Fourier-transformed age structures described in the text. The fraction of the recruitment variance present at a given frequency which should be removed before SR analysis is given by the difference between the squared gain and 1.0.

possible filtering strategies. These examples provide a better intuitive understanding of what filtering theory does for SR analysis because analytic solution to the Fourier transform of the age structure defining stock is available in each case. These solutions clarify the rationale for filtering recruitment data before doing SR analysis.

Model I: Recruitment Independent of Population Density

The first model to be considered here is one suggested by Evans (1987). Evans proposes that filtering theory be applied to a SR model of the form

(1) Recruitment = constant + noise,

i.e. one where there is no SRR in the normal sense. As Evans notes, "estimating the SRR then means estimating the constant, and is an elementary statistical problem." It is informative to do the formal analysis to see why the biological age structure absent from this model is important to the application of filtering theory to SR analysis.

Biologically realistic models require that recruits have parents and are not spontaneously generated, regardless of the functional relationship established by density-dependent mechanisms. Explicitly defining recruitment as a constant independent of stock size therefore means that past recruitments have no pathway by which they can influence future recruitment levels, no matter how violent the fluctuations in recruitment. This could occur, for example, if the parent stock was in some geographically remote area and local recruitment was from pelagic eggs and larvae transported into the area by advection, as might occur on isolated coral reefs. Local stock and recruitment levels would thus be completely divorced.

There is a critical difference between this model and the assumption frequently used in fisheries that recruitment is roughly independent of parent stock over some range of stock sizes. In the latter model it is assumed that density-dependent processes intrinsic to the population *approximately* decouple stock and recruitment; in Evans' model, recruitment is *unrelated* to stock size because of the specification that recruitment is a constant independent of past recruitment levels. Thus the same recruitment would be obtained even if the local stock size is zero.

It may be that in some fisheries, recruitment is roughly independent of stock size over some range of population densities, but it is critical that this be established empirically, rather than merely being assumed. The collapse of many formerly productive marine populations under exploitation is sufficient evidence to indicate that the assumption is probably wrong.

Filtering theory is a technique for increasing the precision of SR analysis by analyzing the sensitivity of stock to recruitment variation in order to assist in the study of the inverse problem, SR analysis. What form of age structure could underly the absolutely constant recruitment model? None of the past recruitments form the adult stock generating recruitment, so the age-structure coefficients describing how recruitment from x yr ago contributes to forming the current adult stock are all zero, i.e.

$$k(x) = 0, \qquad \text{for all } x.$$

What does application of filtering theory show? The squared gain of the Fourier-transformed age structure defines the fraction of the recruitment variability present at each frequency s that contributes information on SR processes (Welch 1986). The Fourier transform for Evans' model is

$$(2) \quad K(s) = \sum_{-\infty}^{+\infty} k(x) e^{-i2\pi sx}, \qquad i = \sqrt{-1}$$
$$= 0, \qquad \text{for all } s,$$

since $k(x)$ is always zero. Accordingly, the squared gain $|K(s)|^2$ is also zero at all frequencies (Fig. 1).

What does this imply? As the squared gain is uniformly zero, the result says to take 0% of the recruitment variability at each frequency for use in comparing with stock. This means that *all* of the recruitment data should be discarded because at *no* frequency do the data contain information about SR processes when comparing recruitment with the local stock size! Note the difference from Evans' statement, quoted above, that estimating the SRR means estimating the constant. Analysis of the age structure for Model I demonstrates that even the information about the mean, though useful for other purposes, is irrele-

vant to analyzing the SRR. Filtering theory therefore provides the correct answer about what SR information is hidden in the recruitment data from Evans' proposed model: none.

Model II: Semelparity

Analysis of the other extreme situation is also enlightening. Assume a semelparous organism (annual plant, many insects, or a pink salmon) where all individuals of a given cohort mature at the same age b, reproduce once, and then die. In this model:

$$\text{(3)}\quad \text{Recruitment}(t) = k(b) \times \text{recruitment}(t-b) + \text{noise}$$

where $k(b)$ is the reproductive effort at age b, defined as the number of recruits per unit stock. The model can be more generally written as

$$\text{(4)}\quad \text{Recruitment}(t) = \sum_{x=-\infty}^{+\infty} k(x)\,\text{recruitment}(t-x) + \text{noise},$$

since the age structure can be written as

$$\text{(5)}\quad k(x) = 1, \quad x = b$$
$$= 0, \quad \text{otherwise.}$$

The Fourier transform of this age structure is calculated as before:

$$\text{(6)}\quad K(s) = \sum_{-\infty}^{+\infty} k(x)\,e^{-i2\pi sx} = e^{-i2\pi sb}$$

by definition (5) of the reproductive effort distribution for a semelparous organism.

Analytic solution for the squared gain of (5) is possible. Making use of the fact that the squared gain is equal to the sum of the squared real and imaginary parts at each frequency s, and using Euler's formula

$$\text{(7)}\quad e^{-is} = \cos(s) - i\sin(s),$$

then the squared gain can be calculated as

$$\text{(8)}\quad |K(s)|^2 = Re(K(s))^2 + Im(K(s))^2 = \cos^2(2\pi sb) + \sin^2(2\pi sb) = 1{,}0, \quad \text{for all } s$$

independent of frequency (Fig. 1).

What does this result imply? As expected, the stock time series is the same as the recruitment time series lagged b yr because only one year class forms the parent stock. The stock therefore shows equal variability over time. The result (8) demonstrates this because it says that 100% of the recruitment variability present at each frequency s should be used in studying the relationship between stock and recruitment; in other words, the recruitment variability is equally informative at all frequencies about SR processes.

Model III: "Rectangular" Iteroparity

Analysis of the simple models considered to this point has not entailed any real filtering of the sort that is of practical use. This example shows the benefits of filtering more at some frequencies than at others.

Consider an iteroparous organism with the following life history: sexual maturity occurs at age b, and during the next $T = 5$ yr a constant fraction of total reproductive effort is expended. The organism then dies. This life history might occur if the increase in fecundity with age just offsets the decline in survival until sexual senescence occurs, and stock is defined as the population egg production. Alternatively, the model might equally well represent a population whose adults act as predators on the prerecruits, with the increase in *per capita* predation with age just balancing the decrease in numbers with mortality until senescence. In this case the stock would be defined in terms of the cannibalistic adult population.

In either situation the recruitments from $i = 1, 2, \ldots, 5$ yr ago would be added together with equal weighting to form the stock. The age structure thus has the form

$$\text{(9)}\quad k(x) = 1/T, \quad b \le x \le b+T$$
$$= 0, \quad \text{otherwise,}$$

i.e. a rectangular function in time. In words, $T = 5$ previously produced age groups make equal contributions to forming the stock in each year. What is the squared gain of the Fourier transform for this function? Again, analytical solution is possible (cf. Bracewell 1978):

$$\text{(10)}\quad |K(s)|^2 = \left[\frac{\sin(2\pi sT)}{2\pi sT}\right]^2 = \text{sinc}(2\pi sT)^2.$$

The squared sinc function is drawn in Fig. 1. Unlike the previous two examples, the squared gain varies with frequency: it is 1.0 at $s = 0$, zero at integer multiples of $s = 1/T$, and of intermediate and varying values elsewhere.

What interpretation can be placed on this result? The prescription for using the recruitment data is immediately obvious from analysis of the previous two models: all of the recruitment variability at $s = 0$ is relevant to SR analysis (this is the mean of the time series), *none* of the recruitment data at frequencies $s = 0.2$ or $s = 0.4 \cdot \text{yr}^{-1}$ contain useful information, and at other frequencies at most $|K(s)|^2 = \text{sinc}(2\pi sT)^2$ of the recruitment variability contains information of use to SR analysis.

In Fig. 2, I have shown the SR plots obtained when I use the simple SR model:

$$\text{(11)}\quad \text{Recruitment}_{t+1} = C(\text{stock}_t)^D \cdot e^{\text{noise}_t}.$$

Stock is defined using the iteroparous age structure of Model III, i.e. as the weighted sum (9) of the preceding five recruitments, and assuming the Cushing SR model (11). The coefficients of the SR model may be estimated by first log-transforming the stock and recruitment data and then calculating the parameters for the linearized equation. As the environmental "noise" model, I have assumed that

$$\text{(12)}\quad \text{Noise}_t = A\sin(2\pi s_0 T), \quad s_0 = 0.2 \cdot \text{yr}^{-1},$$

i.e. the "noise" varies sinusoidally at precisely that frequency where stock is totally unresponsive to recruitment variability. This example is artificial because external variability is only present at one frequency, but it clearly illustrates the benefits of filtering out the variability in the recruitment data which is uninformative about the SR processes.

By varying A, the relative importance of the density-independent processes to recruitment can be made arbitrarily large.

TABLE 1. Parameter estimates and standard errors (in brackets) obtained for the data sets graphed in Fig. 2. Parameter estimates have been obtained by linear regression after log-transforming the stock and recruitment data.

	Exponent, D	Intercept, ln C[a]	
	Estimate (SE)	Estimate (SE)	R^2
True value	0.667 (—)	−0.916 (—)	—
	A = 1.0		
Unfiltered	0.774 (1.277)	−0.751 (0.402)	0.518
Filtered	0.650 (0.042)	−0.934 (0.175)	0.998
	A = 0.8		
Unfiltered	0.742 (0.901)	−0.787 (0.351)	0.635
Filtered	0.654 (0.036)	−0.932 (0.197)	0.999
	A = 0.6		
Unfiltered	0.716 (0.616)	−0.823 (0.308)	0.758
Filtered	0.657 (0.032)	−0.930 (0.214)	0.999

[a]ln(0.4) = −0.916.

A physical basis for this model might be the response of recruitment to some oceanographic variable having a well-defined periodicity, say upwelling of nutrients induced by one of the nodal components in the astronomical tide. Although this model is obviously highly idealized, it allows a very graphic example of how filtering can remove noise, improve the precision of parameter estimates, and reduce the width of confidence intervals. Monte Carlo studies of the benefits of filtering are reported in Welch (1987a) for more realistic noise models.

The effects of filtering are shown in Fig. 2 for a SR model with $C = 0.75$ and $D = \frac{2}{3}$, so that both recruitment and total population are collapsing from the initial equilibrium towards a new equilibrium population size of $C^{\frac{1}{1-D}}$, or C^3 for the present example. The model therefore mimics the situation occurring when a fishery develops and exceeds the initial compensatory capacity of the SRR.

The recruitment data have been filtered after log-transformation according to the squared gain of the rectangular age structure (10), using the frequency domain technique described in Welch (1987a, 1987c). Parameter estimates for the SRR calculated from the log-transformed data are given in Table 1 for three different levels of noise. Which method of analysis gives a more satisfactory picture of the SR dynamics?

Excluding part of the irrelevant information in the recruitment data allows a more unambiguous look at the SRR, an ability purchased at the cost of having to know something about the age structure determining "stock." This ability is desirable, since otherwise it is very difficult to argue that fishing exceeds the capacity of the SRR to maintain population size within economically viable limits (compare the standard errors on the parameter estimates). Thus, while the example is admittedly contrived, since density-independent noise will generally be distributed across all frequencies, it clearly illustrates the benefits of removing the uninformative recruitment variability prior to analyzing the SR data.

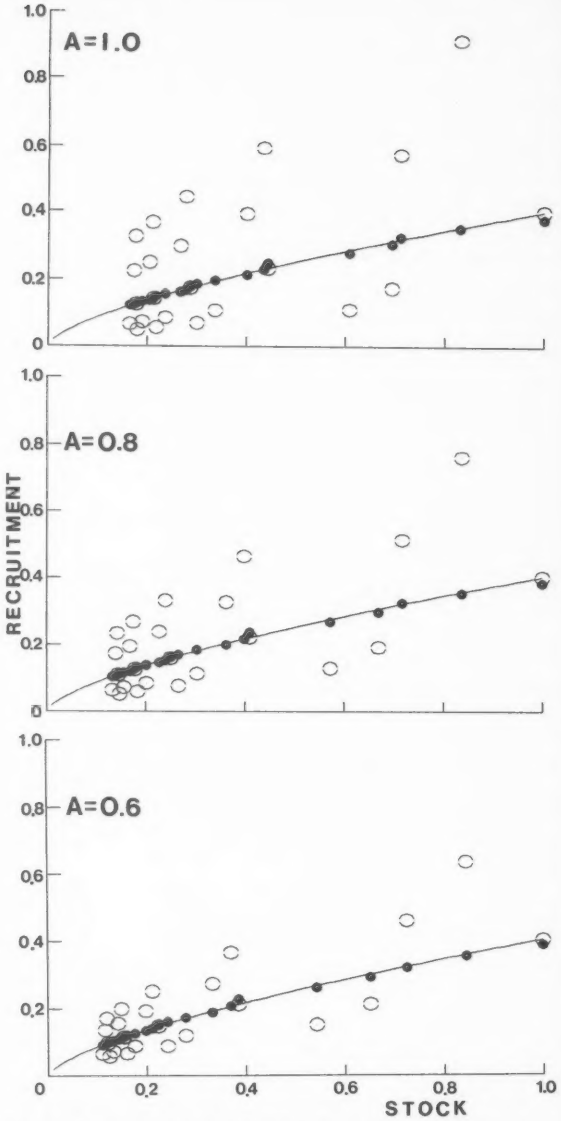

FIG. 2. SR plots before and after filtering (open and closed circles, respectively), assuming the iteroparous age structure of Model III with $C = 0.4$ and the magnitude of the "environmental" contribution to recruitment scaled by A, indicated. For this very special case where all of the density-independent noise can be identified by filtering theory, exact identification of the true SRR is possible. Filtering has been done using the frequency domain method described in Welch (1987a) after log-transformation.

Discussion

Three models of the SRR have been analyzed. In the first model, recruitment is totally independent of stock size. In the second model, recruitment is dependent on the abundance of a stock formed of one age group. In the third model, recruitment is dependent on the abundance of a stock formed equally by several age groups.

For each model, filtering theory provides a useful guide to the information content of the recruitment data, as well as a recipe for excluding that part of the data irrelevant to the study of the SRR (Welch 1986, 1987b). As the numerical example provided with the last model demonstrates, the ability to filter out the irrelevant noise can be critical to the precise determination of the form of the SRR when data are limited and extrinsic influences on recruitment are large, as is frequently the case.

A further use of filtering theory, as yet unexplored, lies in the analysis of environmental influences on recruitment. By using just the recruitment variability excluded by filtering an investigator can increase his or her confidence that the cause of the recruitment variability studied is external, and not generated by fluctuations in stock size.

Age structure based filtering is thus not merely an abstract technical tool. As well as significantly increasing the probability that SR parameter estimates are close to the expected values (Welch 1987a), examination of the stock and filtered recruitment plot can also help to improve an investigator's judgement of what kind of curve is best fit to the data. The choice of the appropriate measure of *stock* is, however, outside the purview of filtering theory. Once a measure has been settled on, the theory can be used to evaluate and extract the SR information content of recruitment data, but it is not a substitute for careful throught beforehand about the biologically appropriate model for stock. If the measure of stock chosen is inappropriate, then so will the application of filtering theory; but so will the SR analysis done without the benefit of filtering theory. Thus, more care in choosing "stock" is called for than has been the case in the past.

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References

BRACEWELL, R. N. 1978. The Fourier transform and its applications. 2nd ed. McGraw-Hill Book Co. Ltd., New York, NY. 444 p.

EVANS, G. T. 1987. Filtering recruitment time series: comment. Can. J. Fish. Aquat. Sci. 44: 691–692.

WELCH, D. W. 1986. Identifying the stock–recruitment relationship for age-structured populations using time-invariant matched linear filters. Can. J. Fish. Aquat. Sci. 43: 108–123.

1987a. Frequency domain filtering of age-structured population data. Can. J. Fish. Aquat. Sci. 44: 605–618.

1987b. Use and precision of age structure based recruitment filtering theory: reply to Evans. Can. J. Fish. Aquat. Sci. 44: 692–694.

1987c. Efficient frequency domain filtering algorithm for small data sets. Can. Tech. Rep. Fish. Aquat. Sci. (In press)

Potential of *Eimeria sardinae* (Apicomplexa: Eimeridae) Oocysts for Distinguishing between Spawning Groups and between First- and Repeat-Spawning Atlantic Herring (*Clupea harengus harengus*)

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McGladdery, S. E. 1987. Potential of *Eimeria sardinae* (Apicomplexa: Eimeridae) oocysts for distinguishing between spawning groups and between first- and repeat-spawning Atlantic herring (*Clupea harengus harengus*). Can. J. Fish. Aquat. Sci. 44: 1379–1385.

Prevalence of *Eimeria sardinae* oocysts was closely correlated with the maturity stage of the testes of Atlantic herring (*Clupea harengus harengus*). Prevalence was low in testes of immature fish, increased in ripe and spawning fish, and decreased in postspawning fish. No correlation was found between prevalence and age of spawning herring. The uniformly high prevalences in mature fish indicated the efficiency of transmission on the spawning grounds, where infective oocysts are released. Infection of first-spawning herring (approximately age 3) indicated that the oocysts may be dispersed to surrounding areas or immature fish may associate with spawning aggregations. Therefore, this parasite could not be used to distinguish first from repeat spawners. Prevalence of *E. sardinae* peaked in May and September, and possibly in June and early July, thereby distinguishing two, and possibly three, spawning groups. A previous study indicated no correlation between maturity stage and infections by *E. sardinae* in northeastern Atlantic herring. The difference between the two sides of the Atlantic is attributed to greater mixing of immature and adult herring around spawning grounds and/or greater dispersal of infective oocysts from spawning grounds in the northeastern Atlantic, compared with those in the northwest.

La prévalence des oocystes de *Eimeria sardinae* présentait une forte corrélation avec le stade de maturité des testicules de hareng de l'Atlantique (*Clupea harengus harengus*). La prévalence était faible dans les testicules des poissons immatures, augmentait chez les poissons matures en frai et diminuait après le frai. Aucune corrélation n'a été notée entre la prévalence et l'âge des harengs en frai. Les prévalences uniformément élevées chez les poissons matures indiquaient l'efficacité de la transmission dans les frayères, où les oocystes infectieux sont

libérés. L'infection des harengs frayant pour la première fois (environ d'âge 3) indiquait que les oocystes pouvaient être dispersés dans les zones avoisinantes ou que des poissons immatures se joignaient aux groupes de géniteurs. Ce parasite ne pouvait donc servir à distinguer les poissons frayant pour la première fois des autres. La prévalence de *E. sardinae* atteignait des maximums en mai et en septembre et, peut-être, en juin et au début de juillet, indiquant ainsi la présence de deux ou, peut-être, de trois groupes de géniteurs. Une étude antérieure n'avait pas permis d'établir de corrélation entre le stade de maturité et l'infection par *E. sardinae* chez le hareng du nord-est de l'Atlantique. L'auteur attribue cette différence entre les deux côtes de l'Atlantique à un plus important mélange des harengs immatures et adultes dans les environs des frayères et/ou à une plus grande dispersion des oocystes infectieux à partir des frayères du nord-est de l'Atlantique, comparativement à celles du nord-ouest.

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One of the most persistant problems currently facing herring biologists from the Gulf of St. Lawrence (NAFO Division 4T) is differentiation between spring- and autumn-spawning herring (*Clupea harengus harengus*) and the determination of the degree of mixing of these two groups. Additionally, a technique to determine the level of recruitment of first-spawning fish into each spawning group has yet to be established.

At certain times of year, herring from different spawning groups cannot be distinguished solely by maturity stage. For example, herring overwintering at maturity stage III (see Parrish and Saville (1965) for maturity stage definitions) may spawn in either spring or autumn. The same problem applies to herring at maturity stage III during summer feeding aggregations. Cleary et al. (1982) listed 12 out of 54 maturity stage and month combinations that could not be assigned conclusively to one particular spawning group. In addition, many herring exhibit borderline gonad characteristics, especially between postspawning stage VIII and prespawning stage III, and changes in the gonads due to freezing further complicate assignment of maturity stages (Martin and Chouinard 1985).

The life cycle of the sporozoan *Eimeria sardinae* is closely linked to the reproductive dynamics of male herring: the infective oocyst stage is released with the sperm during spawning, and subsequent infection occurs directly via ingestion of these oocysts on and around the spawning grounds (Lom 1970). The prevalence and development of *E. sardinae* oocysts were, therefore, investigated to determine whether, or not, they could provide information that would help separate maturity stages and spawning groups as McGladdery and Burt (1985) speculated.

An earlier investigation by Kabata (1963) on *E. sardinae* infection of herring collected from northeastern Atlantic waters showed no correlation between the presence of this parasite and the reproductive dynamics of their hosts. This is in direct contrast with the preliminary findings of McGladdery and Burt (1985). The present investigation was, therefore, also aimed at determining why *E. sardinae* showed a correlation with the reproductive dynamics of herring on one side of the Atlantic and not on the other.

Materials and Methods

Between 1982 and 1985 a total of 1889 male herring were collected from various locations around the east coast of Canada (see McGladdery and Burt 1985, fig. 1). The testes were examined for *E. sardinae* by taking a sample of tissue (approximately 0.5 g) from the posterior third of the left testis, placing it on a slide with a drop of physiological saline, and squashing it with a coverslip until the tissue was approximately one cell thick. Preliminary examination of 100 male herring (McGladdery 1984) revealed no significant difference in level of infection between right and left testis. Additional samples (four per testis) from various areas of the testes showed a more or less uniform distribution. Since only zygote and oocyst stages could be readily detected by the tissue squash technique used in this and previous studies, the present investigation was based solely on observation of early and late oocyst stages. Early oocysts were defined as those with no definable sporocysts or with sporocysts showing no schizont formation and fully developed oocysts as those with sporocysts containing fully formed schizonts. The early oocysts were labelled "unsporulated" and the fully developed oocysts "sporulated."

Infections of sporulated and unsporulated oocysts were recorded and compared with the development stage of the testis (using the eight maturity stages recommended to ICES by Parrish and Saville (1965)). Infections were compared with month of collection for all herring between maturity stages III and VIII to prevent bias introduced by the presence of uninfected immature fish. Infection was also compared with age (determined by otolith) of ripening and spawning herring (maturity stages IV–VI), again to prevent bias introduced by immature or postspawning herring. Sample sizes for different maturity stages, ages, and months varied significantly, so all comparisons were based on prevalence (the percentage of infected individuals per sample). Abundance (mean number of parasites per host) was not used because field counts, using the squash technique, were not considered consistent enough to be accurate.

Significant differences in the prevalence of infection were tested among samples (for maturity stage, age, and month of collection) using the chi-square test, and all significant differences referred to in the text have a probability (p) value of less than 0.05.

Results and Discussion

Distinguishing between spawning groups of herring — To distinguish between spawning groups of herring, *E. sardinae* oocysts should, ideally, show differential prevalences which are directly correlated with the reproductive stages of their hosts. In addition, they should not be destroyed or deformed by freezing and thawing prior to examination. Although prevalences of *E. sardinae* in relation to maturity stage differed between years, the overall pattern of infection was similar (Fig. 1). Any differences were considered negligible and a

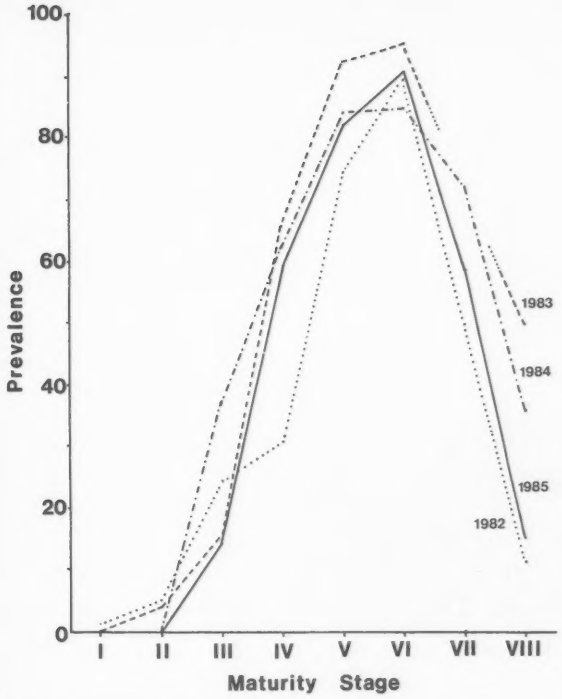

FIG. 1. Prevalence of *E. sardinae* oocysts at each maturity stage for each year between 1982 and 1985.

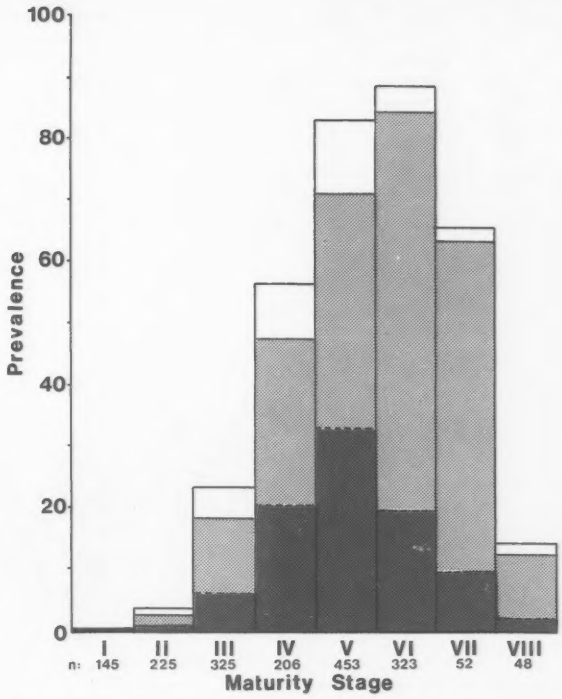

FIG. 2. Prevalence of all *E. sardinae* oocysts (open), fully sporulated oocysts (light stipple), and unsporulated oocysts (heavy stipple) at each maturity stage. Results for 1982–85 are combined.

reflection of the variable sample sizes of each maturity stage; therefore, results for 1982–85 were combined (Fig. 2).

Figure 2 clearly shows the close correlation between the prevalence of *E. sardinae* oocysts and the eight maturity stages defined by Parrish and Saville (1965). Prevalence increased as the gonads matured (stages III and IV), peaked in ripe and spawning herring (stages V and VI, respectively), whilst in postspawning fish (stages VII and VIII) it decreased significantly. Stage VIII gonads develop into stage III to repeat the annual cycle of maturation. The lowest prevalences (less than 5%) were found in immature herring (stages I and II).

The highest prevalence of unsporlated oocysts was found in prespawning herring, followed by a decrease in spawning fish. As would be expected, this decrease corresponded to the peak in prevalence of sporulated oocysts. Some unsporulated oocysts found in stage VIII herring may have been left behind after spawning, evidence for which was the concurrent presence of sporulated oocysts and the degenerate nature of the cyst walls. Other oocysts were, however, uniformly unsporulated, indicating that these fish may not have been infected from their own spawning grounds, but from oocysts released from herring spawning at other times of year, in which instance *E. sardinae* could not develop fully before sperm release. Another possibility, however, is that the fish were actually early resting stage III misclassified as stage VIII because they showed no evidence for prespawning fasting; alteration of gonadal appearance due to freezing and thawing, which can result in such misclassification (Martin and Chouinard 1985), is difficult to detect without detailed histological examination. Infection from another spawning group and/or freezing alteration of the maturity stage characteristics, however, do not appear to be common phenomena, since the differential prevalences relating to the spawning stage of the fish were consistent. A greater homogeneity of prevalence would have been expected if there was appreciable cross-transfer of infection between spawning and nonspawning fish, as discussed later when comparing differences in infection of northeastern and northwestern Atlantic herring.

The low prevalence of *E. sardinae* oocysts in stage III herring, compared with that in prespawning fish at stages IV and V, is unlikely to be an indication of the absence of *E. sardinae*, but rather a reflection of the difficulty of detecting the schizont stages of the life cycle using the squash technique. The significant increase in prevalence between stages III and IV provides further evidence for the close correlation between development of *E. sardinae* and the reproductive dynamics of their hosts. Stage III herring developing towards stage IV would be expected to show some oocyst development, whilst those just recovering from stage VIII would be expected to show none. Obviously the ideal situation is not reflected completely by the samples, but the overall pattern is clearly shown.

Having established this correlation, the question arose of how this could be used to distinguish between different spawning groups? If maturity stages and prevalence of *E. sardinae* oocysts were so closely correlated, why not simply use maturity stage, which can generally be defined by gross observation alone? Unfortunately the durations of maturity stages are, for the most part, unknown and vary considerably between individual stocks (Iles 1964). At certain times of year, therefore, a specific maturity stage may belong to either a spring- or autumn-spawning fish (Table 1).

TABLE 1. Spawning groups most probably encountered for each month of the year based on maturity stage of the gonads at the time of capture. Dashes indicate periods and maturity stages when spawning types cannot be determined. The spawning types in parentheses are possible but not definite. A = autumn spawning; S = spring spawning. (Adapted from Cleary et al. 1982)

Maturity stage	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
I	—	—	—	—	—	—	—	—	—	—	—	—
II	—	—	—	—	—	—	—	—	—	—	—	—
III	—	—	—	A	A	A(S)	A(S)	S	S	A(S)	A(S)	A(S)
IV	—	—	—	S	S	A(S)	A	A	A	A(S)	S	S
V	—	—	—	S	S	S	A	A	A	A	A	A
VI	—	—	—	S	S	S	A	A	A	A	A	A
VII	—	—	—	S	S	S	A(S)	A	A	A	A	A
VIII	—	—	—	A(S)	S	S	S	A(S)	A	A	A	A

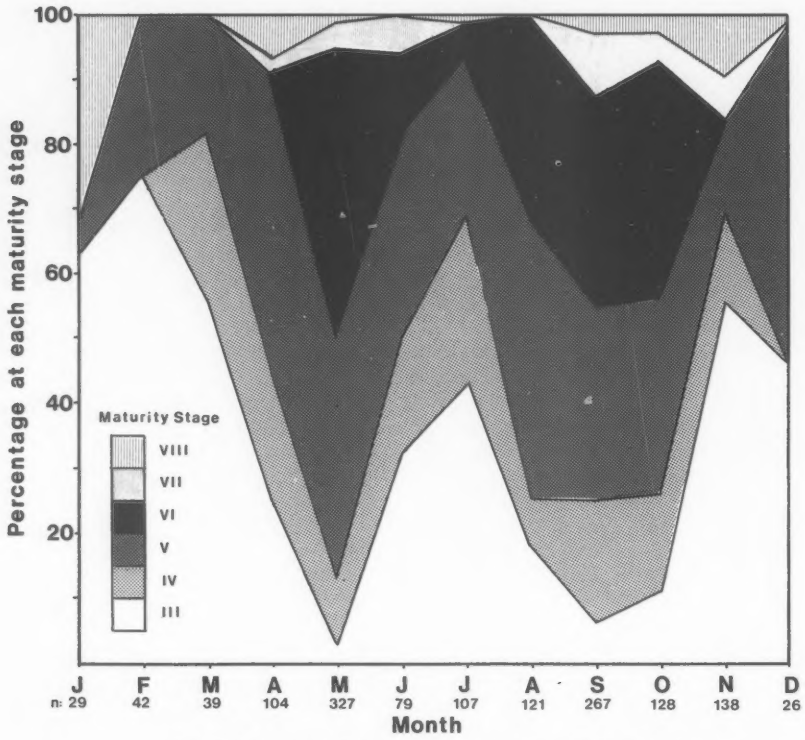

FIG. 3. Percentage of herring at each maturity stage for each month.

The proportion of resting stage III fish clearly decreased in May (corresponding to peak spring spawning, as indicated by the sharp increase in stage VI) and in September (corresponding to autumn spawning and the increased proportion of stage VI) (Fig. 3). A third peak in prevalence of stage VI herring occurs between spring- and autumn-spawning fish in late July, and since there is no similar high prevalence of stage VI fish prior to spring spawning in May, it is believed that this group of spawning fish is unrelated to autumn-spawning herring and constitutes a third spawning group, previously noted by Messieh and MacDougall (1984).

When this pattern was compared with the prevalence of *E. sardinae* oocysts in the same fish for each month (Fig. 4), the results appeared similar. Peaks in prevalence of sporulated oocysts occur in May and September, corresponding to spring and autumn spawning, respectively. Evidence for a third, summer-spawning group of herring is the high proportion of unsporulated oocysts in May and June (when sporulated oocysts would be expected) and the high prevalence of sporulated oocysts in July, 2 mo prior to the expected autumn-spawning increase.

From these results it appears that patterns tentatively indicated solely by maturity stage can be reinforced by the information provided by *E. sardinae* infections. Mixing of spawning groups, as indicated by prevalences around 50%, appears most common in April, prior to spring spawning, in

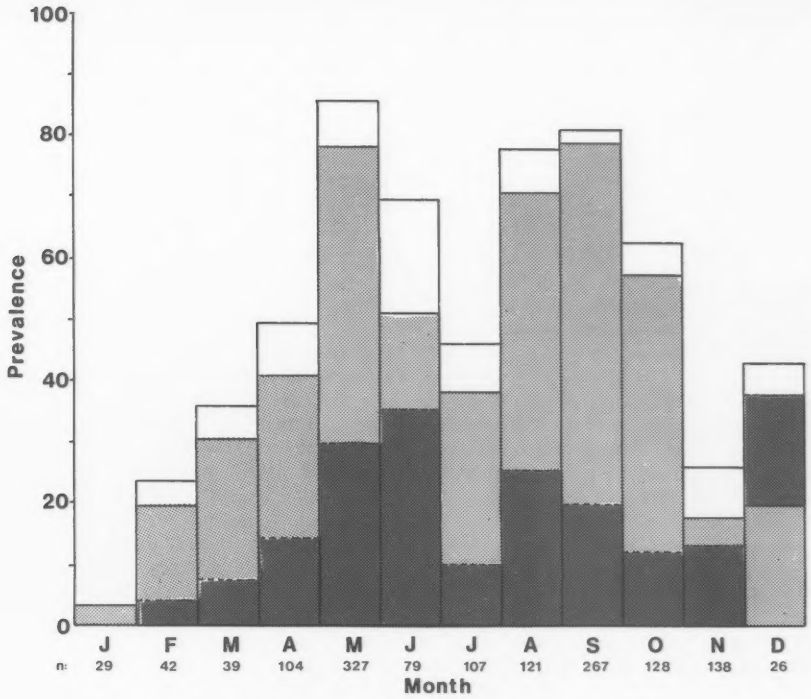

FIG. 4. Prevalence of *E. sardinae* oocysts. See Fig. 2 for key.

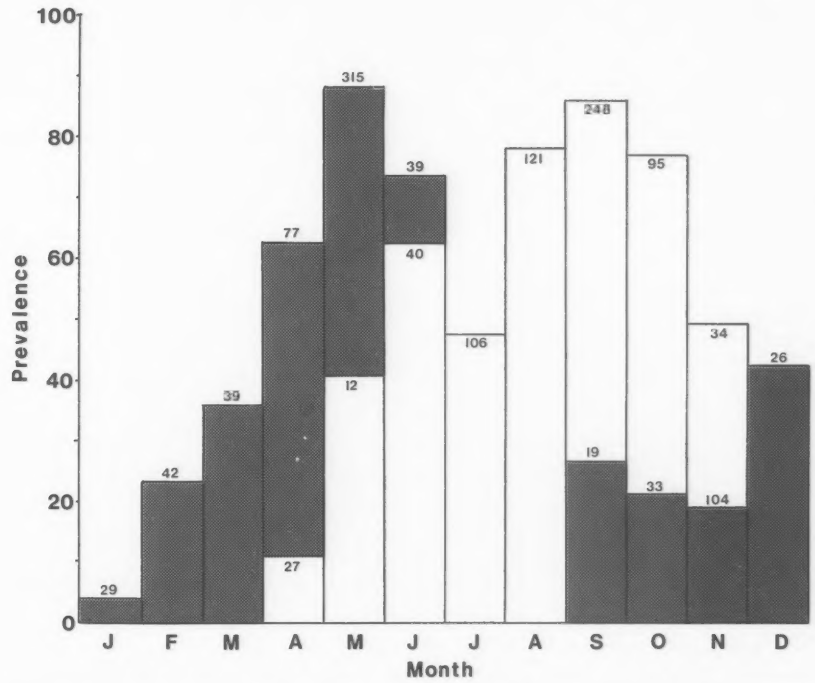

FIG. 5. Prevalence of all *E. sardinae* oocysts in spring- (stippled) and autumn-spawning (open) herring (as defined by maturity stage and date of collection in Table 1).

July, and again in December, following autumn spawning. These results lend support to those derived by Messieh and Tibbo (1971) using morphometrics and meristics.

When spring and autumn spawners were separated using the divisions of Cleary et al. (1982) (Fig. 5), there was a clear peak in prevalence of *E. sardinae* in "spring spawners" in May and a relatively high prevalence in overwintering fish. "Autumn spawners" showed a clear peak in September and October, plus another less well defined peak in June, which appears to belong to a separate summer-spawning group, not found by Cleary et al. (1982). No mixing is indicated in July or December, using maturity stage and date of collection as a definition of spawning group, and only slight mixing was indicated in April. This reinforces the usefulness of *E. sardinae* for providing supplementary information to indicate the discreteness of each spawning stock.

With further work on the dynamics of all the life history stages of *E. sardinae* in male herring, the potential of this parasite as a detailed indicator of the reproductive dynamics of its host increases further. Subdivision of the oocyst stages could provide more detailed definitions of the reproductive stage of the herring, and comparison of results from fresh and frozen fish would serve to determine whether or not *E. sardinae* would be useful for classifying gonads, where the maturity stage is difficult to determine solely from gross morphology.

The technique of preparing tissue is rapid and could easily be incorporated into the laboratory procedure for collection of management data. Detection and definition of the oocysts is easy and does not require extensive parasitological expertise.

Distinguishing between first and repeat spawners — In order for *E. sardinae* oocysts to be of use in separating first spawners from repeat spawners, transmission should ideally occur exclusively on the spawning ground. Juvenile herring are not believed to mix extensively with the spawning fish, and as a result, infection should only affect spawning fish. If this is the case, then a comparison of prevalence of *E. sardinae* oocysts and age of spawning herring should reveal a plateau in prevalence 1 yr following complete recruitment into the spawning population.

No such correlation (for maturity stages IV, V, and VI) was found. All ages of spawners, including age 3 fish, which are definitely first spawners (Messieh 1975), showed high prevalences of infection (range 32–91%). There are a number of possible reasons for this uniform infection of spawning fish. The two most probable are that the infective oocysts released with the sperm may have been dispersed further from the spawning grounds than previously assumed (McGladdery and Burt 1985). The oocysts may have been dispersed by the activity of the spawning fish, turbulence of the water over shallow spawning grounds, or even the early release of sperm due to forced spawning (induced by fishing pressure) near to, but not on, the actual spawning ground. An alternative explanation may be the presence of juvenile herring, which would not be collected in the commercial samples, near or actually on spawning grounds during, or immediately following, spawning.

Whatever the reason for the uniform infection of spawning fish, it is obvious that *E. sardinae* cannot be used to distinguish between first- and repeat-spawning herring.

Differences in infection of northeastern and northwestern Atlantic herring — In contrast with the correlation found between the prevalence of *E. sardinae* oocysts and the reproductive dynamics of northwestern Atlantic herring, no such correlation was found in northeastern Atlantic herring by Kabata (1963). Although there was a distinct increase in prevalence between stages III and VI (Kabata 1963, fig. 10), stage VI herring showed only 40% infection, whilst 80–90% of northwestern Atlantic stage VI herring were infected (Fig. 2). The highest prevalence found by Kabata occurred in stage VIII fish, which was in direct contrast with the decreased prevalence found at this stage in northwestern Atlantic herring (Fig. 2). Another interesting difference between the pattern of infection on both sides of the Atlantic was that immature herring (stage I and II) examined by Kabata showed higher prevalences of *E. sardinae* oocysts than mature, stage III fish. The reason for this apparent anomally is unclear but may be related to the low prevalence found in stage VI herring which indicates that the level of reinfection on northeastern Atlantic spawning grounds was lower than on northwestern Atlantic spawning grounds. This may simply reflect the overall lower prevalence of infection in spawning fish; however, it may also indicate a rapid dispersal of the infective oocysts away from the spawning grounds. This would explain the relatively high level of infection found in juvenile herring, which do not associate directly with spawning fish. The high infection of stage VIII northeastern Atlantic herring may indicate an asynchronous development of oocysts picked up by autumn spawners around spring-spawning grounds. Nursery, feeding, and spawning grounds were known to overlap in the North Sea, where Kabata collected his samples (Parrish and Saville 1965), and expanding on that hypothesis, the greater biomass of herring present in the northeastern Atlantic in the 1960's would have been expected to increase mixing of feeding and spawning aggregations more than at the reduced biomass of herring present today on both sides of the Atlantic (Hubold 1978; Messieh 1984).

Comparison of month of collection and prevalence of *E. sardinae* in autumn-spawning northeastern Atlantic herring (Kabata 1963, fig. 9) revealed a peak overwinter followed by a decline in spring and a low over summer. This is in complete contrast with the pattern found in northwestern Atlantic autumn spawners (Fig. 5) where the peak in prevalence occurred in late summer and early autumn. Again, the contrast in results from both sides of the Atlantic most likely reflects the different nature of spawning and nonspawning population interaction on both sides of the Atlantic as well as the difference in herring biomass present during the two investigations. In order to test the accuracy of this hypothesis, however, a reinvestigation of *E. sardinae* infection of northeastern Atlantic herring is obviously required.

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References

CLEARY, L., J. J. HUNT, J. MOORES, AND D. TREMBLAY. 1982. Herring Ageing Workshop, St. John's, Newfoundland, March, 1982. Can. Atl. Fish. Sci. Advis. Comm. Res. Doc. 82/41: 10 p.

HUBOLD, G. 1978. Variations in growth rate and maturity of herring in the North Sea in the years 1955–1973. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 172: 154–163.

ILES, T. D. 1984. The duration of maturation stages in herring. J. Cons. Int. Explor. Mer 29: 166–188.

KABATA, Z. 1963. Incidence of coccidioses in Scottish herring (*Clupea harengus* L.) J. Cons. Int. Explor. Mer 28: 201–210.

LOM, J. 1970. Protozoa causing diseases in marine fishes. *In* S. F. Snieszko [ed.] A symposium on diseases of fishes and shellfishes. Am. Fish. Soc. Spec. Publ. No. 5: 101–123.

MARTIN, G., AND G. A. CHOUINARD. 1985. Changements de longueur et des stades de maturité du hareng (*Clupea harengus*) dû à la congélation. Can. Atl. Fish. Sci. Advis. Comm. Work. Doc. 85/223: 10 p.

MCGLADDERY, S. E. 1984. Studies of the parasite fauna of Atlantic herring (*Clupea harengus* L.) from the northwestern Atlantic Ocean. Ph.D. thesis, University of New Brunswick, Fredericton, N.B. 286 p.

MCGLADDERY, S. E., AND M. D. B. BURT. 1985. Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Can. J. Fish. Aquat. Sci. 42: 1957–1968.

MESSIEH, S. N. 1975. Maturation and spawning of Atlantic herring (*Clupea harengus harengus*) in the southern Gulf of St. Lawrence. J. Fish. Res. Board Can. 32: 66–68.

1984. Recent changes in fishing effort and catch rates of inshore herring fisheries in the southern Gulf of St. Lawrence. Can. Atl. Fish. Sci. Advis. Comm. 84/59: 34 p.

MESSIEH, S. N., AND C. MACDOUGALL. 1984. Spawning groups of Atlantic herring in the southern Gulf of St. Lawrence. Can. Atl. Fish. Sci. Advis. Comm. 84/65: 28 p.

MESSIEH, S. N., AND S. N. TIBBO. 1971. Discreteness of Atlantic herring (*Clupea harengus harengus*) populations in spring and autumn fisheries in the southern Gulf of St. Lawrence. J. Fish. Res. Board Can. 28: 1009–1014.

PARRISH, B. B., AND A. SAVILLE. 1965. The biology of the Northeast Atlantic herring poplations. Oceanogr. Mar. Biol. Annu. Rev. 3: 323–373.

ANNOUNCEMENTS / AVIS

Fourteenth Annual Aquatic Toxicity Workshop

This workshop will be held November 1–4, 1987, at the Delta Chelsea Inn, 33 Gerrard St. West, Toronto, Ontario. These annual workshops on aquatic and environmental toxicology cover topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of water quality criteria.

For more information on the workshop, please contact: Gary Westlake, Ontario Ministry of the Environment, Rexdale Laboratories, Resources Road, Box 213, Rexdale, Ont. M9W 5L1. Telephone: (416) 235-5797.

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L'atelier susmentionné aura lieu du 1er au 4 novembre 1987 au Delta Chelsea Inn, 33, rue Gerrard ouest, Toronto (Ontario). Ces ateliers annuels sur la toxicologie aquatique et environnementale couvrent des sujets allant de la toxicologie aquatique de base aux applications dans le domaine de la surveillance de l'environnement, de l'établissement de règlements et de lignes directrices et de l'élaboration de critères pour la qualité de l'eau.

Pour de plus amples informations, au sujet de l'atelier, veuillez communiquer avec : Gary Westlake, Ministère de l'Environnement de l'Ontario, Laboratoires Rexdale, Ch. Resources, C.P. 213, Rexdale (Ont.) M9W 5L1. Téléphone : (416) 235-5797.

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